OLYMPUS®

User's Manual Set

FLUOVIEW
FV1200

Biological Confocal Laser Scanning Microscope
FV10-ASW

Notice

Thank you for your purchase of Olympus microscope at this time.

Retain this manual in an easily accessible place near a system for future reference.

AX8274

OLYMPUS®

User's Manual
[Laser Safety Guide]

FLUOVIEW
FV1200MPE
FV1200

Multi Photon Laser Scanning Microscope
Biological Confocal Laser Scanning Microscope

FV10-ASW

Notice

Thank you for your purchase of Olympus microscope at this time.

Retain this manual in an easily accessible place near a system for future reference.

AX8258

IMPORTANT

CAUTION

FV1200MPE is a CLASS 4 laser product; FV1200 is a CLASS 3B laser product.
The procedures for using this system are classified as follows:

- Service
  "Service" means any adjustment or repair performed by service personnels who are provided the service training following to the service manual for this system.
  The performance has influence on the feature of this system, and there is a risk which unintended CLASS 3B or CLASS 4 laser light is emitted.
- Maintenance
  "Maintenance" means adjustment or other procedures performed by customers to maintain that this system functions properly.
- Operation
  "Operation" means all performance described in the user's manuals in this system.
  CLASS 3B or CLASS 4 laser light is only emitted from the objective lens during the actual execution.

The User's Manuals of this system consist of the following:
In order to maintain the full performance of this system and ensure your safety, be sure to read these user's manuals and the operating instructions for the laser unit and light source unit before use.

	User's Manual Sets	
	FV1200MPE	FV1200
FV1200MPE / FV1200 User's Manual [Laser Safety Guide]	○	○
FV1200MPE User's Manual [Safety Manual]	○	
FV1200 User's Manual [Safety Manual]	○	○
FV1200MPE User's Manual [Operation / Maintenance Manual]	○	
FV1200 User's Manual [Operation / Maintenance Manual]	○	○
FV1200 FV10-ASW User's Manual [Quick Start]	○	○

Also, we have prepared one service manual for this system as below. Technical personnels who perform the service are required to take the service training.

- FV1200MPE / FV1200 Service Manual

In case of purchasing the laser simultaneously, we have prepared the following manual for the laser.

- MaiTai Series User's Manual [Quick Start]
- Chameleon User's Manual [Quick Start]

In addition, we have prepared one service manual for the laser as below. Technical personnels who perform the laser service require to take the service training.

- MaiTai Series Service Manual
- Chameleon Service Manual

Reproduction, copying or duplication of a part or all of this software and manual is prohibited.

For customers using FV1000MPE or FV1000:
This manual contains the instructions for FV1200MPE / FV1200 and FV-10ASW (Ver.4.0 and later version).
If the instructions for FV1000MPE / FV1000 and (former version than Ver.4.0) are not covered by this manual,refer to the manual for FV1000MPE / FV1000 on your hand.

Registered Trademarks

Microsoft, Microsoft Windows, Excel for Windows are registered trademarks of Microsoft Corporation.
Other brand names and product names are trademarks or registered trademarks of their respective owners.

Caution

If the system is used in a manner not specified by this manual, the safety of the user may be imperiled. In addition, the system may also be damaged. Always use the system as outlined in this instruction manual.

The following symbols are used to set off text in this instruction manual.

CAUTION : Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury or damage to the equipment or other property. It may also be used to alert against unsafe practices.

◎ : Indicates commentary (for ease of operation and maintenance).

1 Laser Safety Guide ⚠

When using the FV1200MPE, a class-4 laser product, or when using the FV1200, a class-3B laser product, laser light dangerous to the human body may be radiated depending on how it is used. Therefore, this product must be used following the instructions described below, with adequate knowledge about laser safety.

FV1200MPE
CLASS 4 laser product (IEC60825-1:2007)

FV1200
CLASS 3B laser product (IEC60825-1:2007)

This product complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No.50, dated June 24, 2007

This device is product that can only be used under control of laser safety officer.
Assign a laser safety officer and ensure safety in accordance with the instruction given by the said laser safety officer.
In addition, please use this device after reading the user's manual of not only this device but also each laser.

CAUTION **"Caution – Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure".**

If there is an unidentified point, please contact it to your local Olympus representative

1-1 Remarks on combination change or modification of the system

FV1200 and FV1200MPE meet standards for product safety, laser safety and the like under circumstances that they are setup by servicemen authorized by our company.
In case any combination change or modification is done on the system once setup and/or any usage that differs from those described in instruction manual and or safety manual is done, it would stray from the state that meet standards for system safety, laser safety and the like. In such a case, it is responsible for customers to comply with those requirements with respect to safety, laser safety and the like once again.

1-2 The laser class and danger level

Class	Danger Level Description
Class 1	The lasers of this class are always safe regardless of the optical systems where they are used.
Class 1M	The lasers are safe just as those of class 1 mentioned above, as long as observations are made with the naked eye. However, the light becomes dangerous when observations are made through an optical unit.
Class 2	This class is for 400-nm to 700-nm visible laser lights. These lights are safe due to the eyes' aversive response to them.
Class 2M	The same as class 2. However, these lights become dangerous when they enter the eye through an optical unit.
Class 3R	Direct beam observation is potentially dangerous.
Class 3B	Direct beam observation is dangerous.
Class 4	The lights of lasers of this class, being able to cause diffused reflection that is deemed dangerous, pose the danger of causing fire as well that of damaging skin. All laser products that can cause upon human bodies an amount of laser light radiation exceeding the accessible emission limit for class 3B.

- When using the FV1200MPE, a class 4 unit, diffused reflection light is also dangerous in addition to direct laser light and specular reflected light.
- When using the FV1200, a class 3B unit, direct laser light and specular reflected light is dangerous.
- When using the FV1200MPE, Laser light is dangerous because it is an invisible near-infrared light.
- Our engineer will be assigned to handle the assembly and setup of FV1200MPE or FV1200. In case a person who is not our engineer exercises any change on the system (disassembly or remodeling) and, if any malfunction, breakage or accident on the system occurs, please be advised that our company will assume no responsibility. Contact Olympus since we, Olympus, will respond to disassembly, move and the like of the system at customer's expense.
- FV1200MPE uses IR pulse laser. If IR pulse laser enters your eye, blindness may occur.
- Do not put any substance having mirror surface close to objective lens.
- Do not put flammable gas or liquid close to the system since it may cause fire.

- Total laser radiation emitted from the FV1200MPE are as follows:
  MPE (Maximum Permissible Exposure): 0.016 J/m^2 (for a single pulse)
  0.00005 J/m^2 (for a single pulse in the pulse train)
  NOHD (Nominal Ocular Hazard Distance): 50 cm in the vicinity of objective lens *
  Exposure duration: 3X10^4 sec
  * Hereafter, this area is called “Laser Hazard Area”.
- Total laser radiation emitted from the FV1200 are as follows:
  MPE (Maximum Permissible Exposure): 1 W/m^2
  NOHD (Nominal Ocular Hazard Distance): 25 cm in the vicinity of objective lens *
  Exposure duration: 3X10^4 sec
  * Hereafter, this area is called “Laser Hazard Area”.

1-3 Safety functions of the FV1200MPE and FV1200

FV1200MPE is provided with the safety measures corresponding to the specifications required for class 4 laser products.
FV1200 is provided with the safety measures corresponding to the specifications required for class 3B laser products.

1 Operation management using a key

These products are provided with a key to activate the power supply unit (FV10-PSU).
When the unit is not in use, the key must be removed and must be kept and managed by the laser safety officer.

2 Radiation-indicating unit (light)

When laser light is emitted from the object lens, the LED of the scanning unit (FV12-SU) is turned on.
When the LED is turned on, use the utmost caution in utilizing this product.

3 Shutting and attenuating the beam

These products are provided with a built-in shutter to prevent the laser light from entering the eye.

4 Light beam path position (When using the FV1200MPE)

The following methods are used to prevent the laser light from entering the eyes:

- When used in combination with the BXWI, this product is designed so that the fluorescent cube placed at the height of the eyes cannot be inadvertently removed.
- When used in combination with the IX81, this product is designed so that the support for transmitted illumination cannot fall, to prevent the customers from looking inadvertently at the laser light from the objective.

These safety measures are ensured in the state in which OLYMPUS delivers the microscopes. Do not attempt to disassembly the microscope or laser unit.

5 Beam stop

This product is designed so that laser light is not inadvertently leaked into the microscope part or the introductory optical system.

- The laser light emitted from the object lens is terminated with the penetration detecting unit (FV10-TD).
- A cover is placed over the entire introductory optical system. (When using the FV1200MPE)
- A metal cap is attached to each of the unoccupied holes of the revolving nosepiece of the microscope to prevent the laser light from coming out through the hole. (When using the FV1200MPE)

These safety measures are ensured in the state in which OLYMPUS delivers the microscopes. Do not attempt to disassembly the microscope or laser unit.

1-4 Requests from OLYMPUS

OLYMPUS would like to take this opportunity to ask the customer to establish a system to ensure laser safety in line with the customer's facility. The items necessary for ensuring laser safety are listed below.

1 Appointing a laser safety officer

Please take actions to ensure laser safety with the laser safety officer at the center of such activities.

1 Duties of the laser safety officer

Maintain safety by considering combination of the following content:

- Planning and implementing measures for preventing damages due to laser light
- Setting and managing laser controlled area
- Managing key and so on which starts laser equipment
- Inspecting and maintaining laser equipment, and preserving record of them
- Inspecting and maintaining protection tool, and observing if it is being used properly
- Conducting labor hygiene education and preserving record of it
- Other items necessary for preventing damages due to laser light

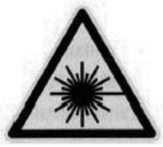
2 Details of the safety education and training

It is requested for the laser safety officer to conduct safety education and training for all of the persons who are to use laser equipment. Such training should include the following:

- Characteristics of laser light and its dangers
- Principles and structures of laser equipment
- How to use laser equipment
- Performance of safety and protective equipment, and how to use them
- Measures to be taken in an emergency and evacuation procedures

2 Laser controlled areas (warning signs and off-limits)

The area where a laser beam of class 3B or above is emitted is defined as laser control area. Define the room where FV1200MPE and FV1200 are set up as laser control area.
Place a warning sign near to each of the laser controlled areas that includes the following information:

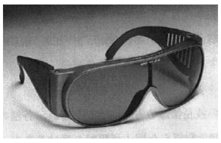


- The name of the laser officer
- Descriptions of the dangers of laser radiation, and cautions about handling
- A sign indicating the installation of laser equipment

3 Preventive eye glasses and protective clothes

In Laser Hazard Area, it is essentially required to wear eye protector and protective clothing. For this reason, in case a microscope should be operated, or a specimen should be set, wear the eye protector.

1 Using protective glasses



Get the protective glasses matching class and wavelength of the laser of this system. In addition, do not look at laser light directly even when wearing protective glasses.

2 Wearing protective clothes (When using the FV1200MPE)

If any work should be done in Laser Hazard Area, wear protective clothing of flame retardant material.

4 Ophthalmologic examination

The persons who use class 4 laser equipment or class 3B laser equipment are requested to have ophthalmologic examinations.
When laser light enters a person’s eye, the person must get an ophthalmologic examination immediately.

1-5 Appendix

1 Reference material

IEC60825-1	“Safety of laser products - Part 1: Equipment classification and requirements”
IEC/TR60825-14	“Safety of laser products - Part 14: A user’s guide”
Food and Drug Administration	“PERFORMANCE STANDARDS FOR LIGHT-EMITTING PRODUCTS”

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OLYMPUS®

User’s Manual
[Safety Manual]

FLUOVIEW
FV1200

Biological Confocal Laser Scanning Microscope
FV10-ASW

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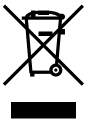
AX8262



In accordance with European Directive 2002/96/EC on Waste Electrical and Electronic Equipment, this symbol indicates that the product must not be disposed of as unsorted municipal waste, but should be collected separately.
Refer to your local Olympus distributor in EU for return and/or collection systems available in your country.

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications.
Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

FCC WARNING: Changes or modifications not expressly approved by the party responsible for compliance-could void the user's authority to operate the equipment.

CALIFORNIA USA ONLY
The touch panel controller of this product uses a Lithium Battery which contains Perchlorate Material -special handling may apply, See www.dtsc.ca.gov/hazardouswaste/perchlorate.

For Korea only

A급 기기 （업무용 방송통신기자재）

이 기기는 업무용(A급) 전자파적합기기로서 판 매자 또는 사용자는 이 점을
주의하시기 바라 며, 가정외의 지역에서 사용하는 것을 목적으로 합니다.

IMPORTANT

CAUTION

FV1200 is a CLASS 3B laser product.
The procedures for using this system are classified as follows:

- Service
  "Service" means any adjustment or repair performed by service personnels who are provided the service training following to the service manual for this system.
  The performance has influence on the feature of this system, and there is a risk which unintended CLASS 3B laser light is emitted.
- Maintenance
  "Maintenance" means adjustment or other procedures performed by customers to maintain that this system functions properly.
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  "Operation" means all performance described in the user's manuals in this system.
  CLASS 3B laser light is only emitted from the objective lens during the actual execution.

The User's Manuals of this system consist of the following:
In order to maintain the full performance of this system and ensure your safety, be sure to read these user's manuals and the operating instructions for the laser unit and light source unit before use.

	User's Manual Sets	
	FV1200MPE	FV1200
FV1200MPE / FV1200 User's Manual [Laser Safety Guide]	○	○
FV1200MPE User's Manual [Safety Manual]	○	
FV1200 User's Manual [Safety Manual]	○	○
FV1200MPE User's Manual [Operation / Maintenance Manual]	○	
FV1200 User's Manual [Operation / Maintenance Manual]	○	○
FV1200 FV10-ASW User's Manual [Quick Start]	○	○

Also, we have prepared one service manual for this system as below. Technical personnels who perform the service are required to take the service training.

- FV1200MPE / FV1200 Service Manual

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If the instructions for FV1000MPE / FV1000 and (former version than Ver.4.0) are not covered by this manual,refer to the manual for FV1000MPE / FV1000 on your hand.

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FLUOVIEW MANUAL CONFIGURATION

The FLUOVIEW system uses two manuals including this “User’s Manual” and the on-screen manual built into the software (“Online Help”).
The User’s Manual is composed of the two following volumes and subject matter:

SAFETY GUIDE

Describes notes and cautions on using the FLUOVIEW system and on types of warning labels.

1 SAFETY CAUTION ⚠ 1
2 WARNING LABELS 9
3 CAUTION FOR SYSTEM OPERATION 26

SPECIFICATIONS

Describes the specifications of the FLUOVIEW system.

1 SPECIFICATIONS 1

Caution

If the system is used in a manner not specified by this manual, the safety of the user may be imperiled. In addition, the system may also be damaged. Always use the system as outlined in this instruction manual.

The following symbols are used to set off text in this instruction manual.

CAUTION : Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury or damage to the equipment or other property. It may also be used to alert against unsafe practices.

◎ : Indicates commentary (for ease of operation and maintenance).

■ PROPER SELECTION OF THE POWER SUPPLY CORD

If no power supply cord is provided, please select the proper power supply cord for the equipment by referring to “Specifications” and “Certified Cord” below;

CAUTION: In case you use a non-approved power supply cord for Olympus products, Olympus can no longer warrant the electrical safety of the equipment.

Specifications

Voltage Rating	125V AC(for 100-120V AC area) or , 250V AC (for 220-240V AC area)
Current Rating	6A minimum
Temperature Rating	60°C minimum
Length	3.05 m maximum
Fittings Configuration	Grounding type attachment plug cap. Opposite terminates in molded-on IEC configuration appliance coupling.

Table 1 Certified Cord

A power supply cord should be certified by one of the agencies listed in table 1, or comprised of cordage marked with an agency marking per Table 1 or marked per Table 2. The fittings are to be marked with at least one of agencies listed in Table 1. In case you are unable to buy locally in your country the power supply cord which is approved by one of the agencies mentioned in Table 1, please use replacements approved by any other equivalent and authorized agencies in your country.

Country	Agency	Certification Mark	Country	Agency	Certification Mark
Australia	SAA		Italy	IMQ	
Austria	ÖVE	ÖVE	Japan	JET,JQA,TUV, UL-APEX/MITI	PSE, T
Belgium	CEBEC	CEBEC	Netherlands	KEMA	KEMA EUR
Canada	CSA	SA	Norway	NEMKO	N
Denmark	DEMKO	D	Spain	AEE	AEE
Finland	FEI	FI	Sweden	SEMKO	S
France	UTE		Switzerland	SEV	+S
Germany	VDE	VDE	United Kingdom	ASTA,BSI	ASTA,
Ireland	NSAI		U.S.A	UL	UL

Table 2 HAR Flexible Cord

APPROVAL ORGANIZATIONS AND CORDAGE HARMONIZATION MARKING METHODS

Approval Organization	Printed or Embossed Harmonization Marking (May be located on jacket or insulation of internal wiring)		Alternative Marking Utilizing Black-Red-Yellow Thread (Length of color section in mm)		
			Black	Red	Yellow
Comite Electrotechnique Belge (CEBEC)	CEBEC	‹HAR›	10	30	10
Verband Deutscher Elektrotechniker (VDE) e.V. Prüstelle	‹VDE›	‹HAR›	30	10	10
Union Technique de d´Electricite´ (UTE)	USE	‹HAR›	30	30	10
Instituto Italiano del Marchio di Qualita´ (IMQ)	IEMMEQU	‹HAR›	10	30	50
British Approvals Service for Electric Cables (BASEC)	BASEC	‹HAR›	10	10	30
N.V. KEMA	KEMA-KEUR	‹HAR›	10	30	30
SEMKO AB Svenska Elektriska Materielkontrollanstalter	SEMKO	‹HAR›	10	10	50
Österreichischer Verband für Elektrotechnik (ÖVE)	‹ÖVE›	‹HAR›	30	10	50
Danmarks Elektriske Materialkontroll (DEMKO)	‹DEMKO›	‹HAR›	30	10	30
National Standards Authority of Ireland (NSAI)	‹NSAI›	‹HAR›	30	30	50
Norges Elektriske Materiellkontroll (NEMKO)	NEMKO	‹HAR›	10	10	70
Asociacion Electrotecnica Y Electronica Espanola (AEE)	‹UNED›	‹HAR›	30	10	70
Hellenic Organization for Standardization (ELOT)	ELOT	‹HAR›	30	30	70
Instituto Portages da Qualidade (IPQ)	| np |	‹HAR›	10	10	90
Schweizerischer Elektro Technischer Verein (SEV)	SEV	‹HAR›	10	30	90
Elektriska Inspektoratet	SETI	‹HAR›	10	30	90

Underwriters Laboratories Inc. (UL)	SV, SVT, SJ or SJT, 3 X 18AWG
Canadian Standards Association (CSA)	SV, SVT, SJ or SJT, 3 X 18AWG

SAFETY GUIDE

On This Volume

This volume describes caution and notes for safe use of the FLUOVIEW FV1200 system. Be sure to read this volume completely before use.

⚠ If the system is operated in a manner not specified by this manual, the safety of the user may be impaired. IN addition, the system may also be damaged. Always operate the system as outlined in this user's manual.

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Registered Trademarks 2

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1 Warning Labels 9

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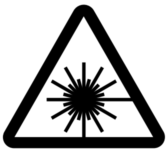
1 SAFETY CAUTION⚠

This product has been specified as a laser product of one of the following classes according to the combined laser.

(1) Visible-light laser combination
CLASS 3B (IEC60825-1:2007)

(1)



LASER RADIATION
AVOID EXPOSURE TO BEAM
CLASS 3B LASER PRODUCT

50mW MAX CW 400-700nm
IEC 60825-1:2007

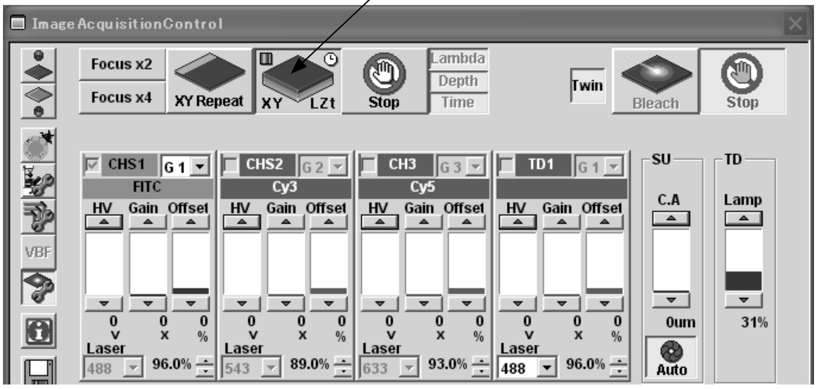
This product complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No.50, dated June 24, 2007

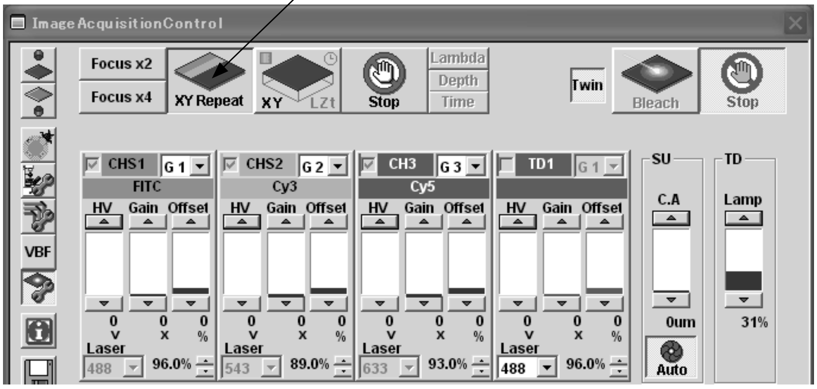
Laser products of Class 3B are products that can only be used under control of laser safety officer.
Assign a laser safety officer and ensure safety in accordance with the instruction given by the said laser safety officer.
In “IEC 60825-1, Safety of laser products − Part 1: Equipment classification and requirements” and “IEC/TR 60825-14, Safety of laser products − Part 14: A user’s guide”, it requires safety preventive measures for users of laser products.
Before using, read IEC 60825-1 and IEC/TR 60825-14 of the latest edition carefully and then, use the products after enforcing adequate measures.
Unless the safety measures indicated in IEC 60825-1 and IEC/TR 60825-14 are provided, our company cannot insure safety of the product since it may cause exposure of dangerous laser emission.

Safety Notes

Take care regarding the following points during operation.

CAUTION **When the displayed function window ([Acquire] panel) shows the condition that the button marked with the arrow is pressed as described in the drawing, the laser beam is being output from the top of objective lens. There is potential danger that the reflected light may enter your eyes or the scattered light may leak outside etc. Take special care for operating. Never look at the specimen while the laser scanning.**


Shows “the laser beam being output”.
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
Twin
Bleach
Stop
CHS1
G1
FITC
CHS2
G2
Cy3
CH3
G3
Cy5
TD1
G1
HV
Gain
Offset
SU
C.A
TD
Lamp
0um
31%
Laser
488
96.0%
543
89.0%
633
93.0%
Auto
VBF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Cy5
TD1
G1
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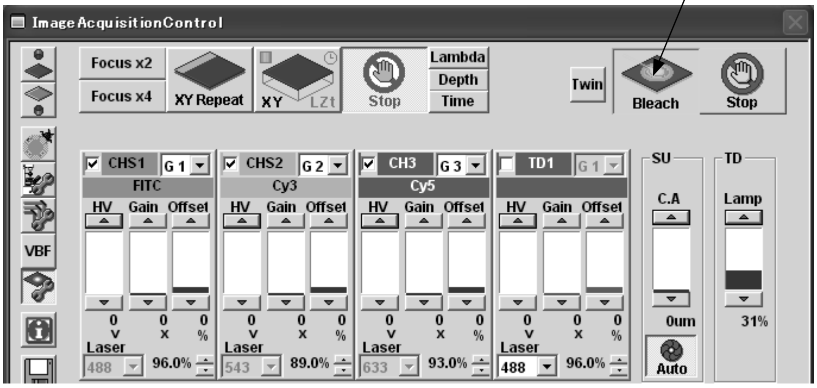
Gain
Offset
SU
C.A
TD
Lamp
0um
31%
Laser
488
96.0%
543
89.0%
633
93.0%
Auto
VBF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Stop
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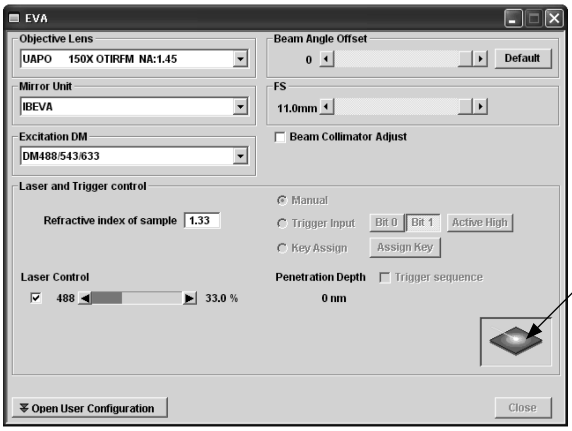
G1
FITC
CHS2
G2
Cy3
CH3
G3
Cy5
TD1
G1
HV
Gain
Offset
SU
C.A
TD
Lamp
0um
31%
Laser
488
96.0%
543
89.0%
633
93.0%
Auto
VBF



Objective Lens: UAPO 150X OTIRFM NA:1.45

Mirror Unit: IBEVA

Excitation DM: DM488/543/633

Beam Angle Offset: 0

FS: 11.0mm

☐ Beam Collimator Adjust

Laser and Trigger control

Refractive index of sample: 1.33

● Manual

○ Trigger Input

○ Key Assign

Laser Control

☑ 488 33.0 %

Penetration Depth: 0 nm

☐ Trigger sequence

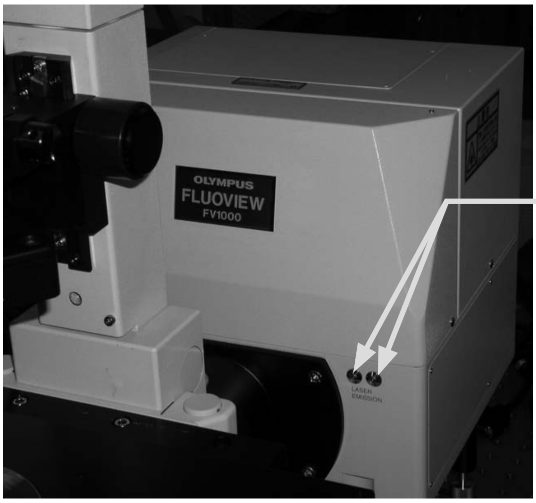
In case of [Manual] mode
“Laser beam being output” appears.
In case of [Trigger Input] or [Key Assign] mode
“Laser beam being output or Laser emission
being waited” appears.

CAUTION

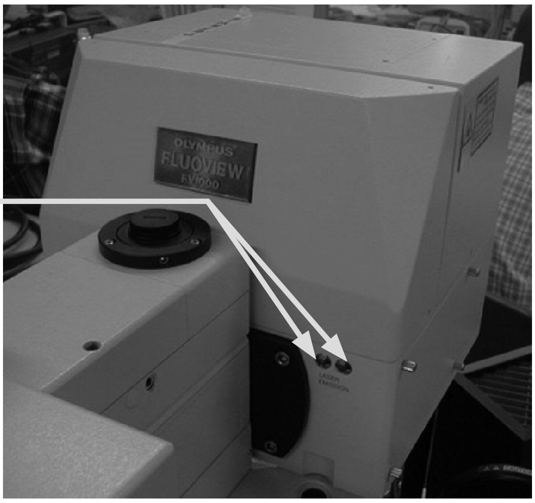
- When [Trigger Input] mode is used and, even if ‹Laser emission› button is set to the state of “Laser beam being output”, the system would remains in the state of “Laser emission being waited” when the trigger is not active. Therefore, the laser is not emitted and it is emitted when the trigger becomes active. When the laser emission should be terminated with [Trigger Input] mode, click ‹Laser emission › button and set the system to the state of laser stop.
- When [Key Assign] mode is used and, even if ‹Laser emission› button is set to the state of “Laser beam being output”, the system would remain in the state of “Laser emission being waited” when the key is not pressed. Therefore, the laser is not emitted and it is emitted when the key is pressed. When the laser emission should be terminated with [Key Assign] mode, click ‹Laser emission› button and set the system to the state of laser stop.

CAUTION **While the LED lamp on the FV12-SU is illuminated, the laser beam is being output from the objective mount hole. There is potential danger that the reflected light may enter your eyes or the scattered light may leak outside etc. Take special care for operating. Never look at the specimen while the laser scanning.**

It indicates that laser is being emitted when two lamps are lit.


OLYMPUS
FLUOVIEW
FV1000
LASER EMISSION
Two lamps

FV12-SU (with FV12-SUIX)


OLYMPUS
FLUOVIEW
FV1000
LASER EMISSION

FV12-SU (with FV10-SUBX)

CAUTION **If any one of the LED lamps is illuminated solely while activating the FLUOVIEW software, there may be a system failure. Stop operating and contact your local Olympus representative.**

CAUTION

- Do not expose your hand or finger to the laser beam output from the objective mount hole, objective tip or condenser lens, or your skin may be damaged. Also, never attempt to output the laser beam outside the system by inserting a mirror or a similar object in the light path. Otherwise, the laser beam may enter your eyes, which is extremely hazardous.

- If the device is breaking down, the laser beam may illuminate under the objective. Do not put your hand in under the objective.
  When you place a specimen on the stage, you should pull out the stage, place a specimen on it and move the specimen toward under the objective with the stage movement knob. To take the specimen off, you should also pull out the stage with the stage movement knob.

- Place the specimen horizontally on the stage. If the specimen inclines, the laser beam may reflect around the microscope, which is extremely hazardous.

- Do not touch the specimen while the laser is emitted. It is very hazardous, since reflection angle of the laser is changed.

- Do not incline the specimen under the objective. Otherwise, the laser beam may reflect, which is extremely hazardous.

- Do not remove the cube turret or the revolving nosepiece for objectives while the software is running.

- Do not set or remove the specimen while the laser beam is output (during laser scanning). Otherwise, the reflected light from the specimen may enter your eyes, which is extremely hazardous. When placing a specimen on the stage, move the stage center out of optical axis with stage movement knob, then place a specimen and bring it under the objective lens by the stage movement knob.

- Never attempt to remove any of the units while the laser beam is output (during laser scanning), the laser beam may be output from the system and cause an extremely hazardous situation. If you want to remove each unit, refer to our service representatives since it is defined as the service activities.
  For detailed operation of lamp replacement, see the ”FV1200 User’s Manual [Safety Guide]”.

- When you do not attach a objective to a the revolving nosepiece for objectives, please be sure to attach a cap.

- Do not touch the stage while it is moving. Otherwise, the stage may move and pinch your hand or finger, which is hazardous.

- If the system is disassembled (for example, opening a cover with a tool), the ability of the product and safety cannot be assured. Never attempt to disassemble the equipment.

- Do not bend or pull the laser fiber cable with an excessive force or step on it. If the laser fiber cable is damaged, the laser light may leak outside it and cause an extremely hazardous situation. Should such an event occurs, immediately turn off the laser power and contact your local Olympus representative.

- The air outlet of the laser cooling fan blows out warm air. Do not place a flammable or non-heat-resistant object near the heat from the air outlet.

- Connect the system to ground correctly. Connect the ground correctly. Otherwise, Olympus can no longer warrant the electrical safety and performance of the equipment.

- In case that the fuse of each unit blows out, please contact our sales office in your local area for replacement.

- Observe Electric Safety Standard of your country for power cable. If the proper cord is not used, product safety and performance cannot be guaranteed.

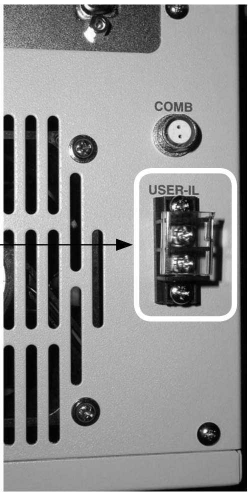
- Do not plug or unplug a cord or cable while the power is supplied. Make sure that the main switch is set to “O” (OFF) before plugging or unplugging a cord or cable.

- The power cord may be melt by the lamp housings, if the cord comes in contact with them. There is potential danger of electric shock. Distribute the power cord at an enough distance from the lamp housings.

- The absolute alcohol used to clean the optics is highly flammable. Take special care in handling against fire, main switch ON/OFF, etc. Room ventilate is recommended while you use absolute alcohol.

- The power supply connector to the HeNe laser is clamped with special hardware, which should never be removed in order to unplug the connector. Even after the laser power switch is set to OFF, prevent electric shock by not touching the connector, because the laser head may still be charged.

- FV10-PSU is equipped with the external interlock connector (Current Rating: 70mA, Voltage Rating: DC 24V). If you want to connect the interlock switch to this connector, please be sure to turn PSU main switch [O](OFF) and remove the short-circuit metal of the connector prior to connecting.



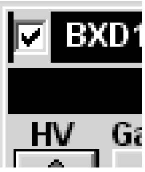
Remote interlock connector of laser

PSU Backside Panel

- Disposal of this product
  The laser tube used with this system should always be disposed of in accordance with your local industrial waste treatment regulations. When disposal is required, please consult your local Olympus representative.
  The FV12-HSD equipped in the FV10MP-BXD-GAP contains arsenic(As) and its compounds. At the time of disposable of this product, consign it to the authorized industrial waste disposer or follow the local rule of industrial waste. When you use this product in other country and dispose it there, follow the rule of industrial waste there.

- Also refer to the section described about safety of the manual of surrounding units.

- Usage note of the external PMT unit (BXD/IXD)
  When image acquisition is not executed, uncheck the [Ch] check box of the [Ch] group box on the [Image Acquisition Control] window.



[Ch] check box

If the check box is left checked under the circumstance it is light in the room, it may damage PMT.

Safety Symbols

The following symbols are found on the system. Study the meaning of the symbols, and always use the system in the safest possible manner.

Symbol	Explanation
♨	Indicates that the surface becomes hot, and should not be touched with bare hands. Otherwise burns may result.
⚠	Before use, carefully read the user's manual. Improper handling could result in injury to the user and/or damage to the equipment.
⚡	Indicates that the voltage is so high. Take special care to prevent electric shock.
✹	Indicates that the laser beam is applied. Take special care in handling.
I	Indicates that the main switch is ON.
O	Indicates that the main switch is OFF.

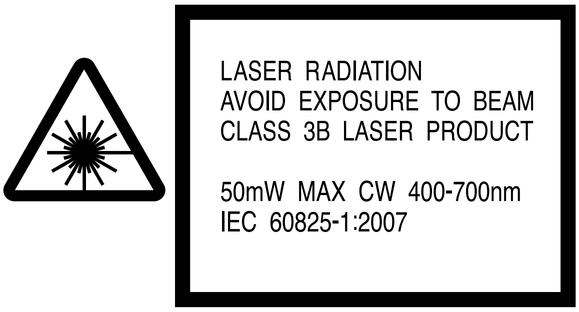
2 WARNING LABELS

2-1 Laser Safety Warning Labels

The kind of Labels attached on the laser system depends on the laser combination you have.

1 Warning Labels

(1)

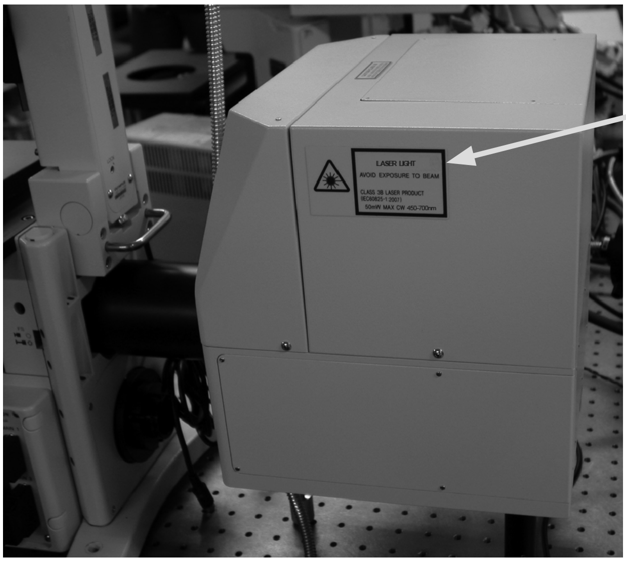


LASER RADIATION
AVOID EXPOSURE TO BEAM
CLASS 3B LASER PRODUCT

50mW MAX CW 400-700nm
IEC 60825-1:2007

Attaching Position

- Combination with IX83, IX81



Label (1) as shown
The right-hand-side of a
Scan Unit.

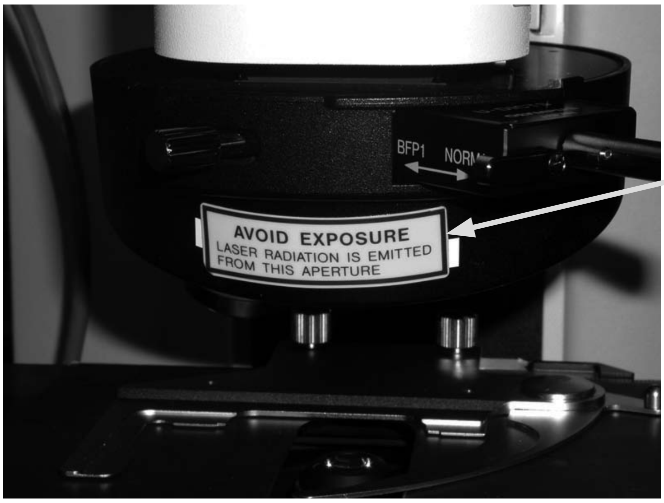
2 Aperture Label

(2)

AVOID EXPOSURE-
LASER RADIATION IS EMITTED
FROM THIS APERTURE

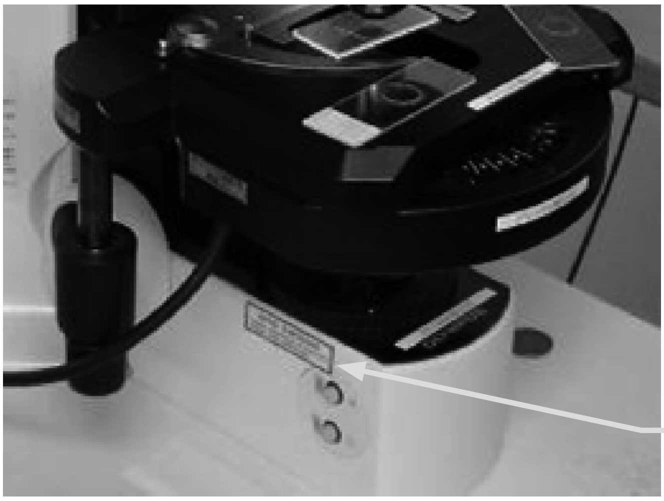
Laser emits from the place with the above labels and indicated with arrow.

- Combination with BX61


BFP1
NORM
AVOID EXPOSURE
LASER RADIATION IS EMITTED
FROM THIS APERTURE

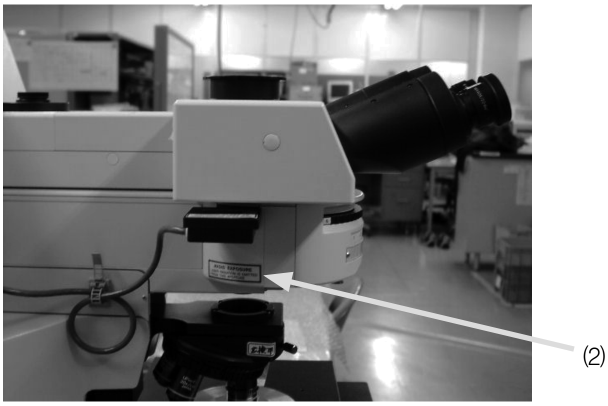
(2) Objective or revolving nosepiece hole

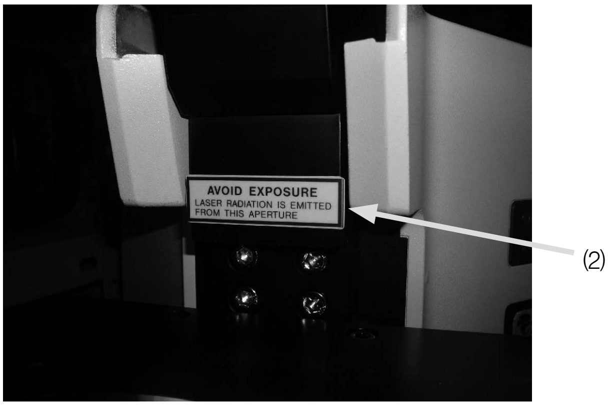
- Combination with BX61, BX61WI



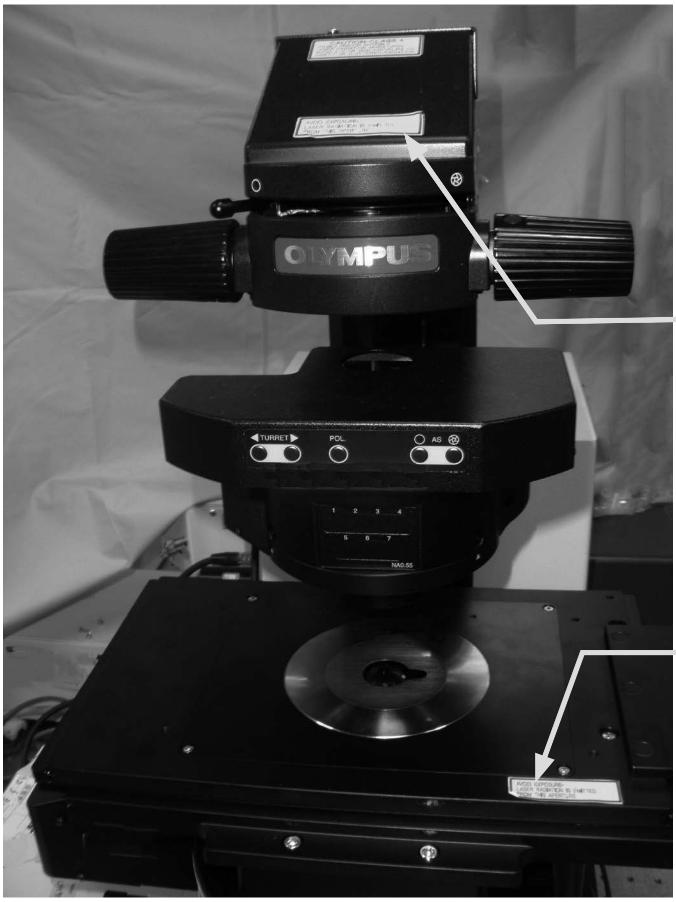
(2)
Condenser lens.

- Combination with BX61WI




AVOID EXPOSURE
LASER RADIATION IS EMITTED FROM THIS APERTURE
(2)

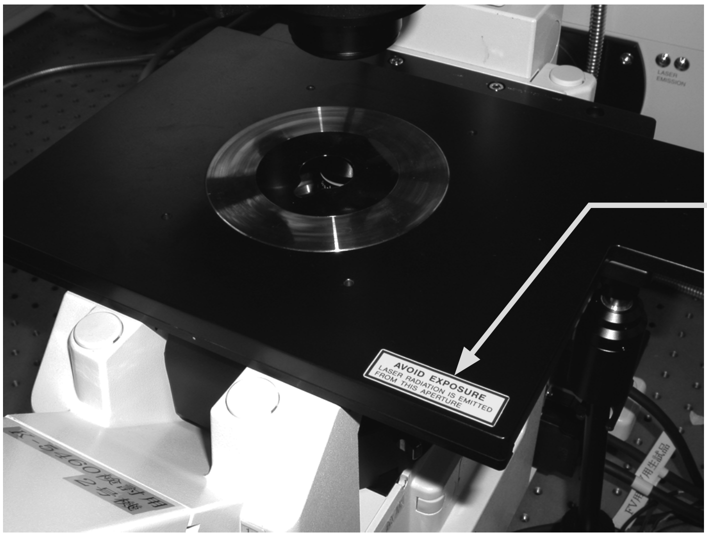
- Combination with IX83


OLYMPUS
TURRET
POL.
AS
1 2 3 4
5 6 7
NA0.55

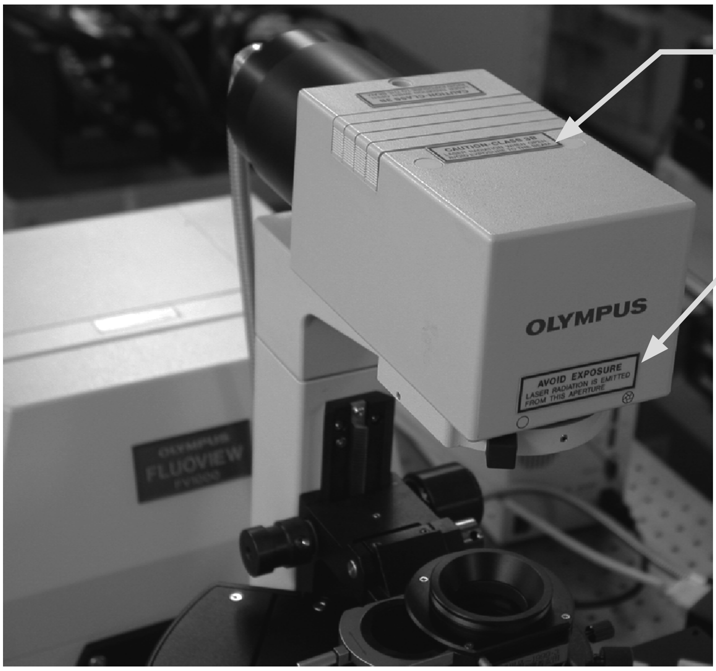
(2)
Condenser lens.

(2)
Objective lens.

- Combination with IX81


AVOID EXPOSURE
LASER RADIATION IS EMITTED
FROM THIS APERTURE

(2) Objective lens.


CAUTION-CLASS 3B
OLYMPUS
AVOID EXPOSURE
LASER RADIATION IS EMITTED
FROM THIS APERTURE
OLYMPUS
FLUOVIEW

(2) Filter holder.

(2) Condenser lens.

3 Protective Housing Labels

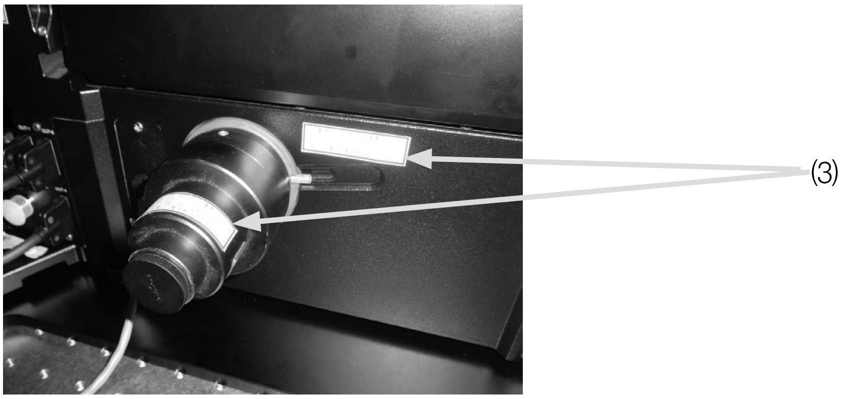
(3)

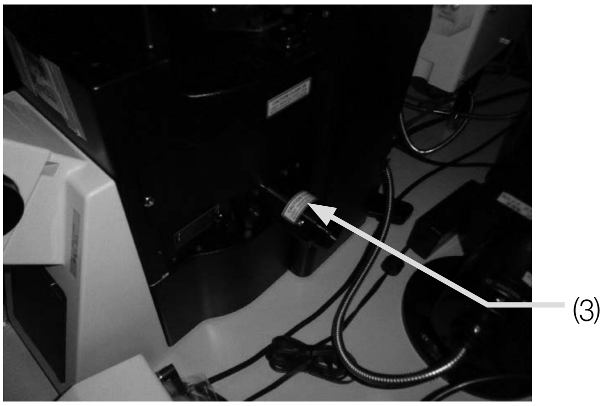
CAUTION-CLASS 2M LASER RADIATION
WHEN OPEN
DO NOT STARE INTO THE BEAM OR VIEW
DIRECTLY WITH OPTICAL INSTRUMENTS

(4)

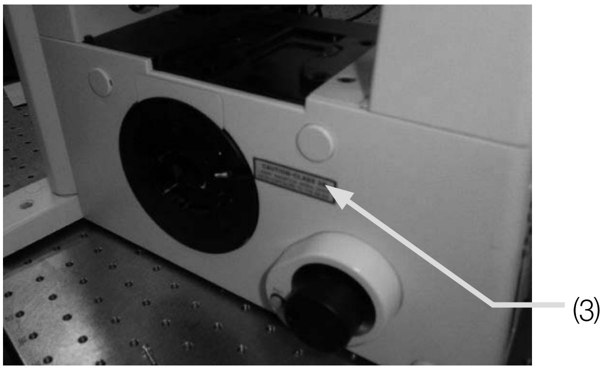
CAUTION-CLASS 3B LASER RADIATION
WHEN OPEN
AVOID EXPOSURE TO THE BEAM

- Combination with IX83

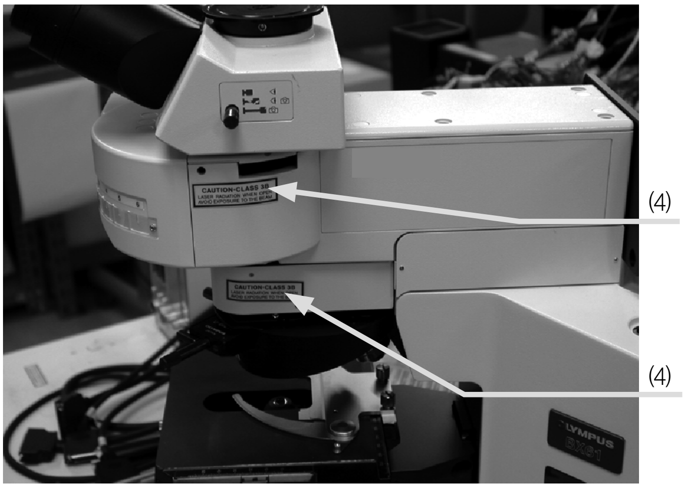




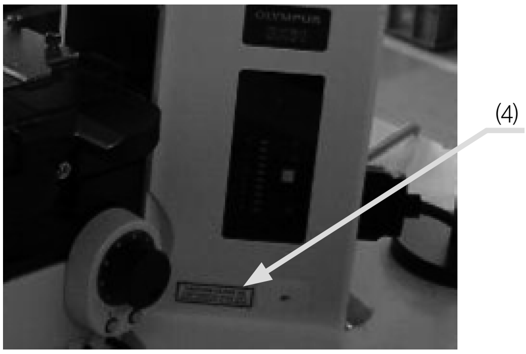
- Combination with IX81(EVA)



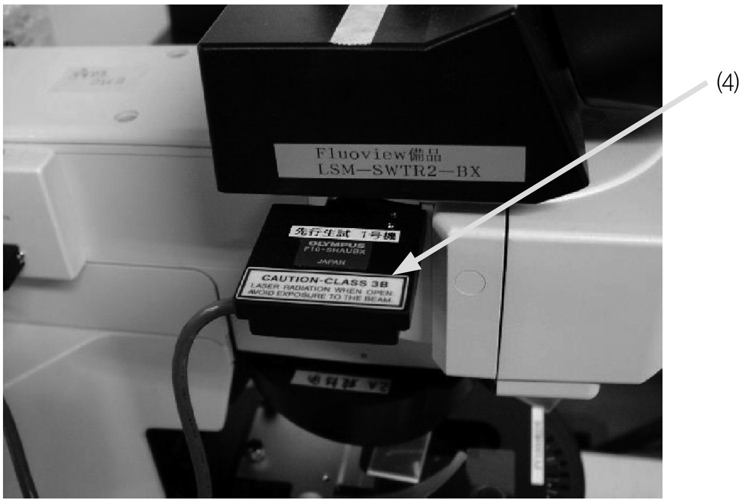
- Combination with BX61, BX61WI


(4)
(4)
CAUTION-CLASS 3B
OLYMPUS
BX61

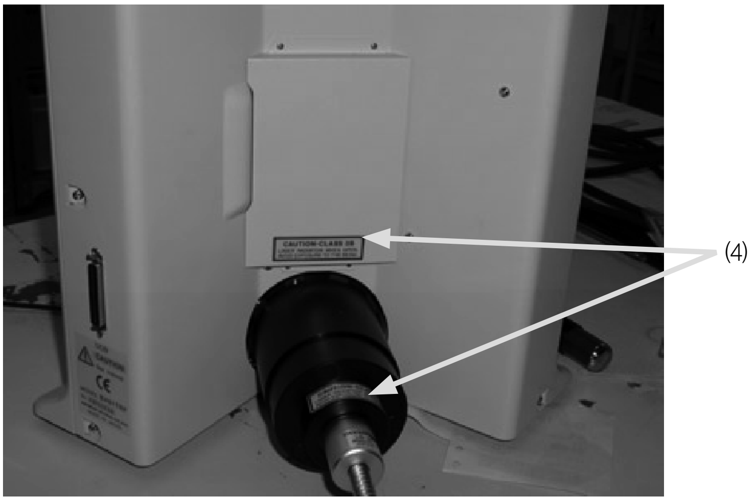
When the transmitted light unit is not installed



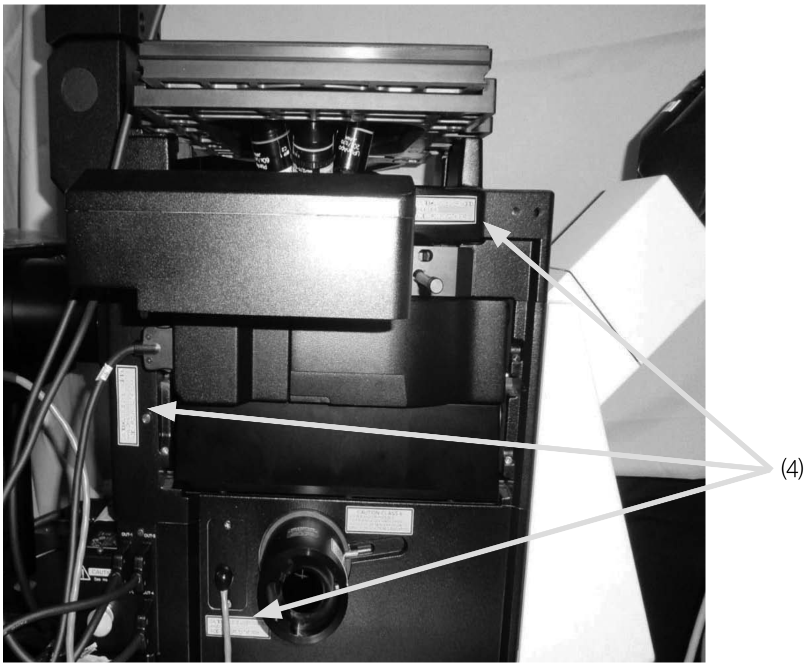
- Combination with BX61, BX61WI

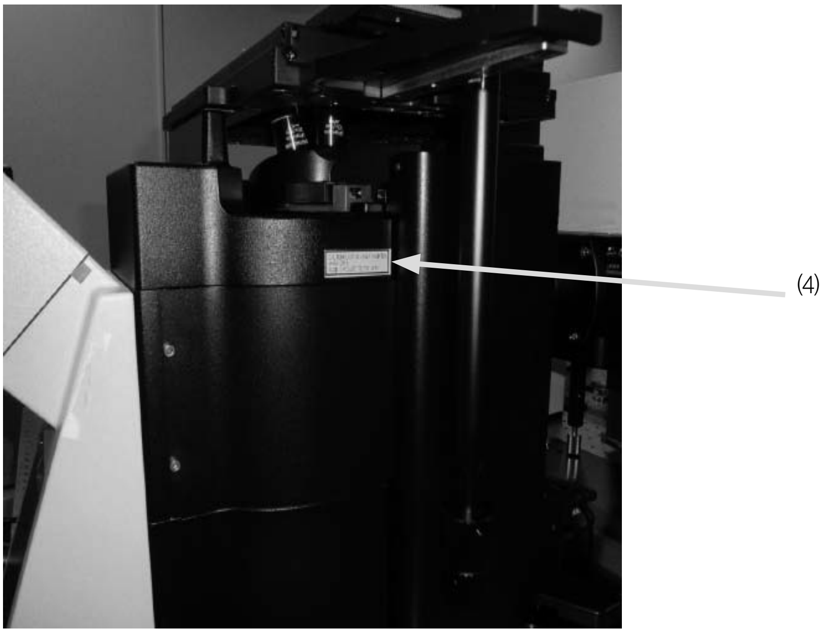

Fluoview備品
LSM−SWTR2−BX
先行生試 1号機
OLYMPUS
JAPAN
CAUTION-CLASS 3B
LASER RADIATION WHEN OPEN
AVOID EXPOSURE TO THE BEAM.
(4)

- Combination with BX61, BX61WI

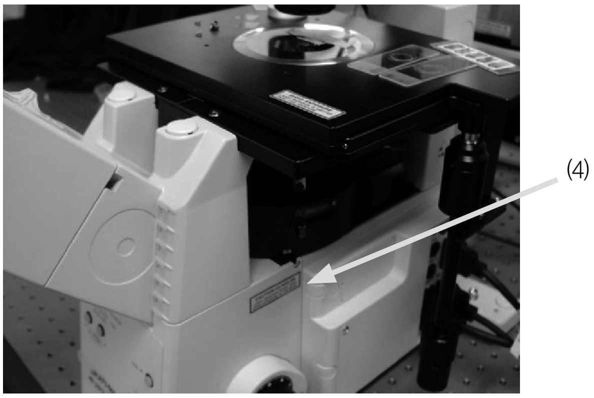

CAUTION-CLASS 3B
(4)

- Combination with IX83

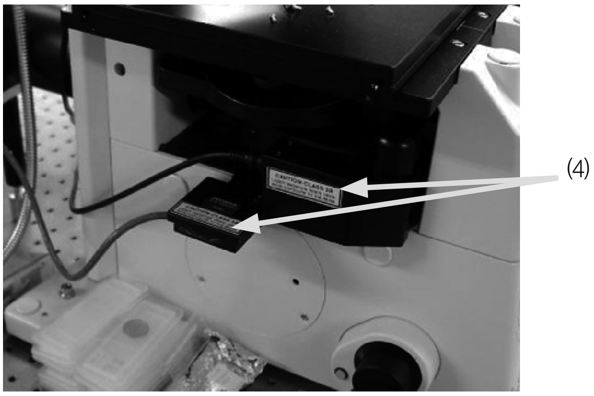




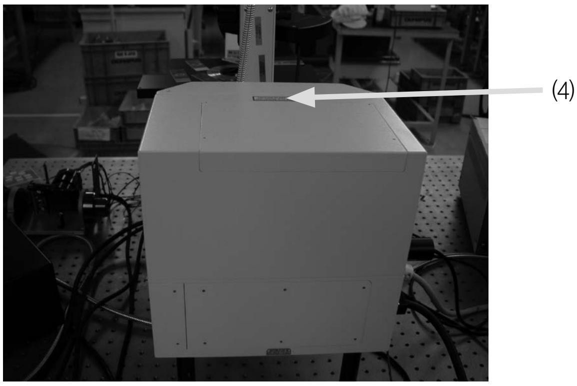
- Combination with IX81



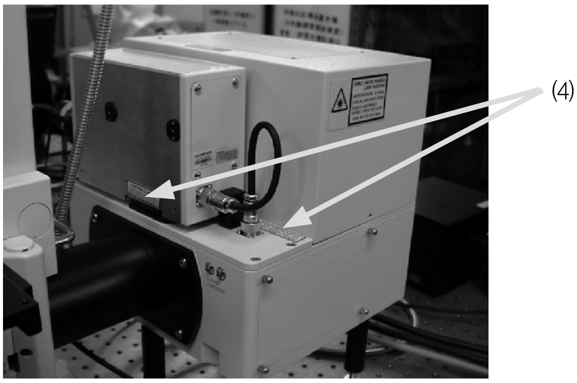
- Combination with IX81



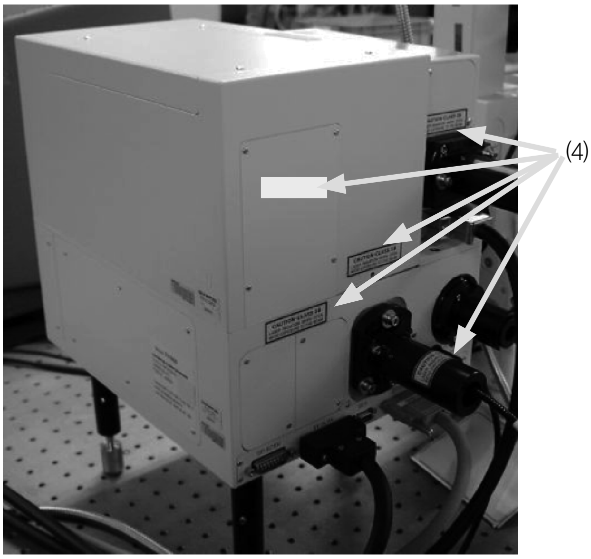
- Combination with BX61, BX61WI, IX83, and IX81



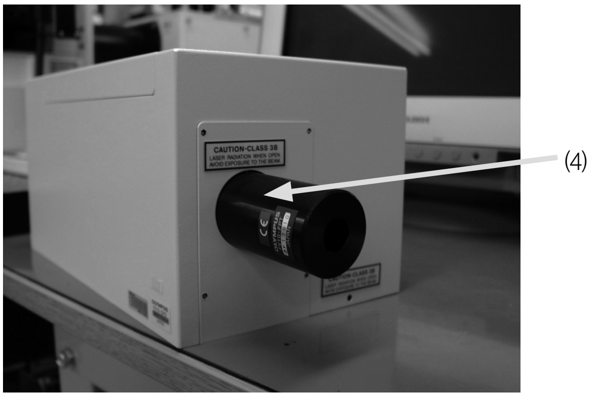
- Combination with BX61, BX61WI, IX83, and IX81



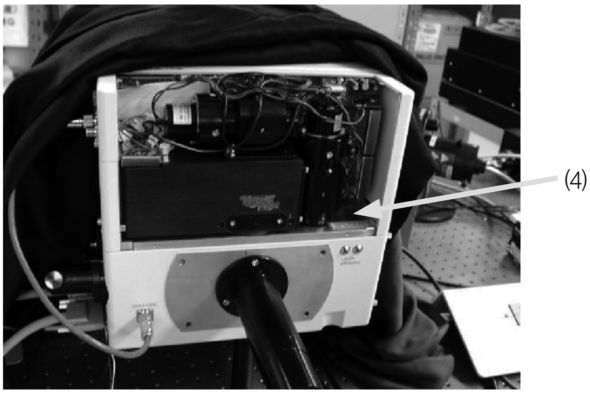
- Combination with BX61, BX61WI, IX83, and IX81



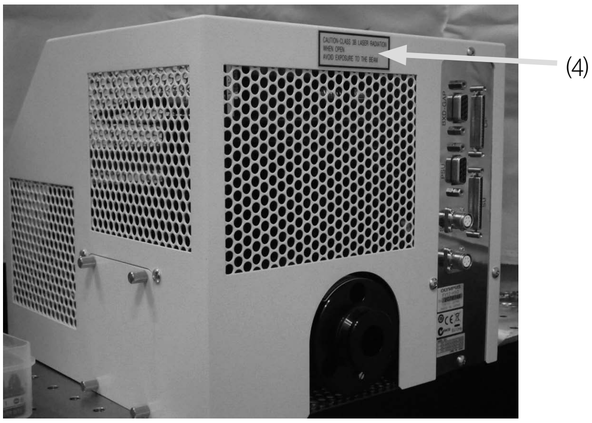
- Combination with BX61, BX61WI, IX83, and IX81


CAUTION-CLASS 3B
LASER RADIATION WHEN OPEN
AVOID EXPOSURE TO THE BEAM
(4)

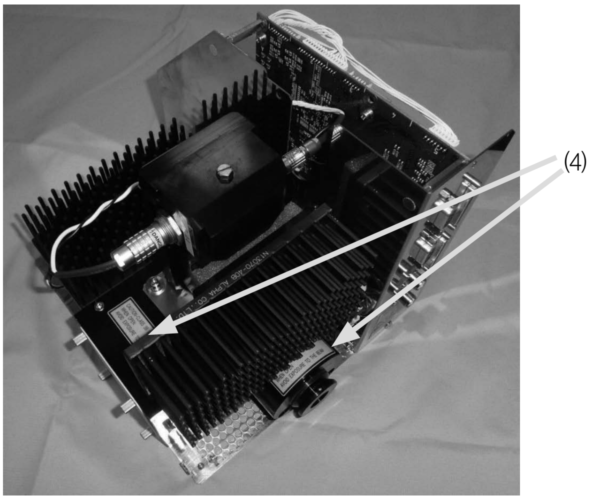
- Combination with BX61, BX61WI, IX83, and IX81



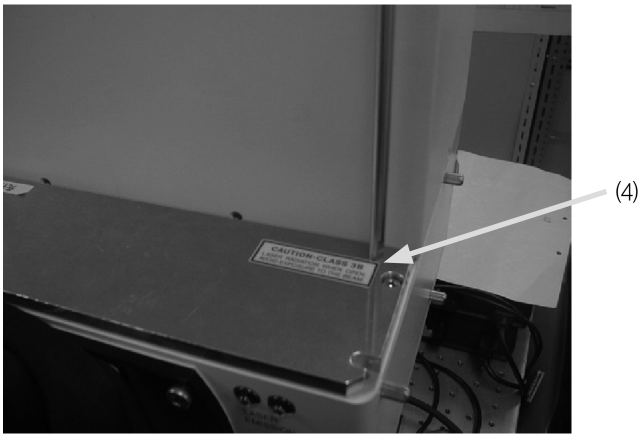
- Combination with BX61, BX61WI, IX83, and IX81



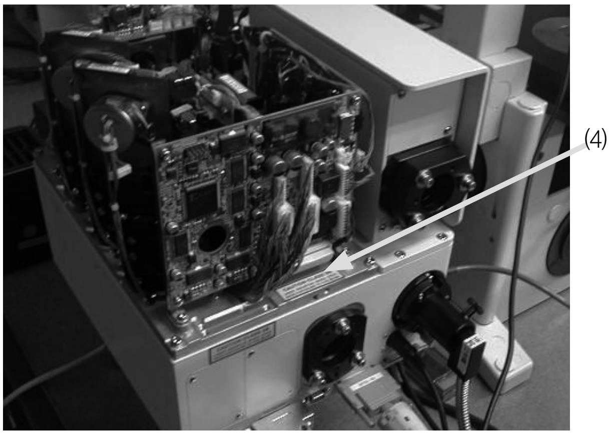
- Combination with BX61, BX61WI, IX83, and IX81



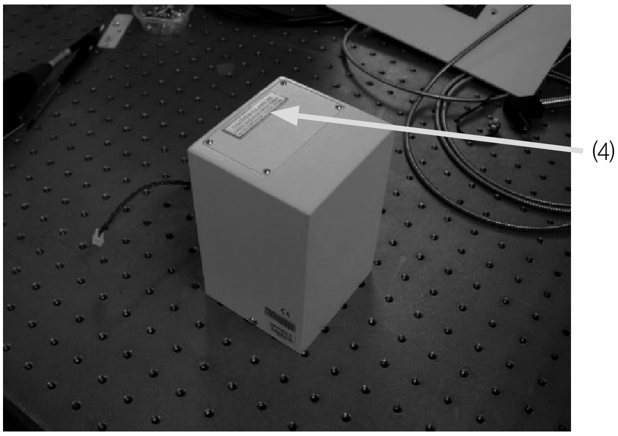
- Combination with BX61, BX61WI, IX83, and IX81



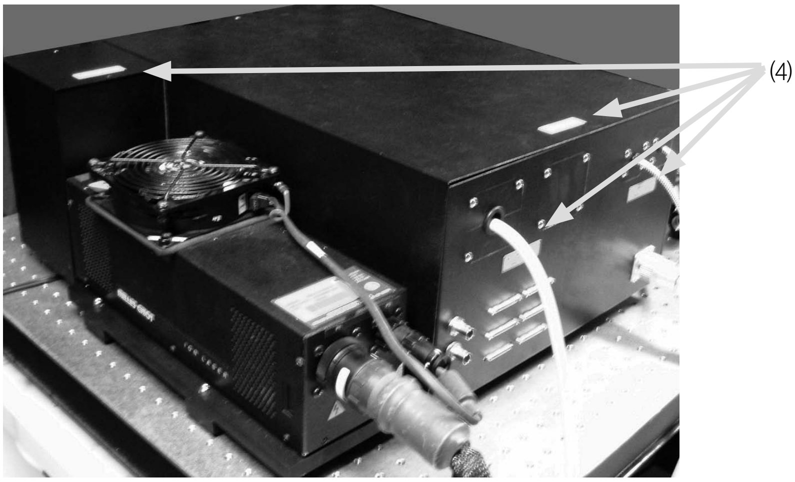
- Combination with BX61, BX61WI, IX83, and IX81



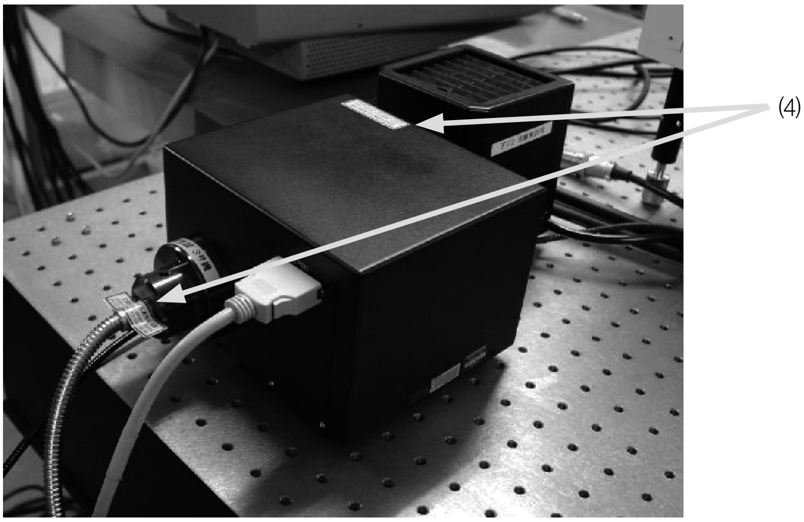
- Combination with BX61, BX61WI, IX83, and IX81



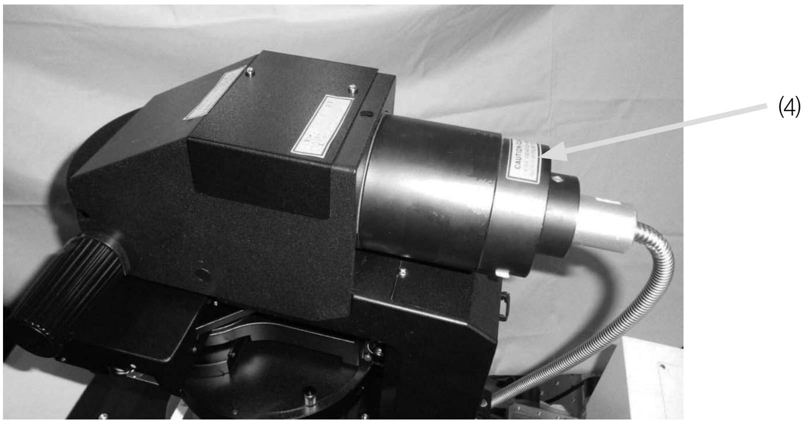
- Combination with BX61, BX61WI, IX83, and IX81



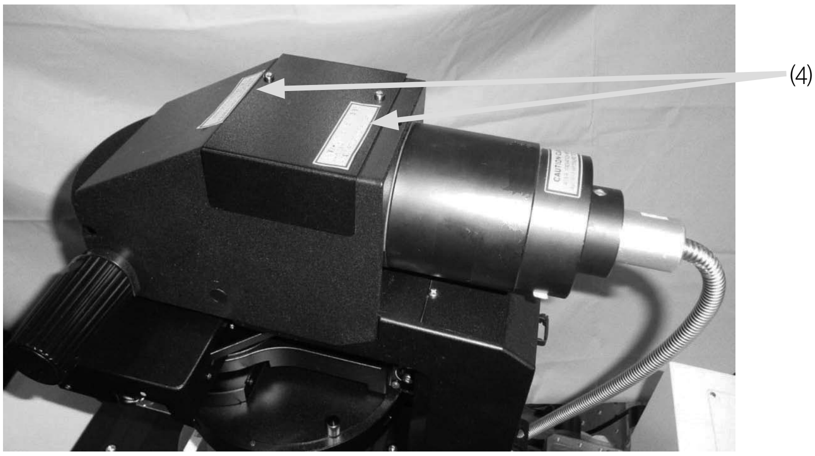
- Combination with BX61, BX61WI, IX83, and IX81



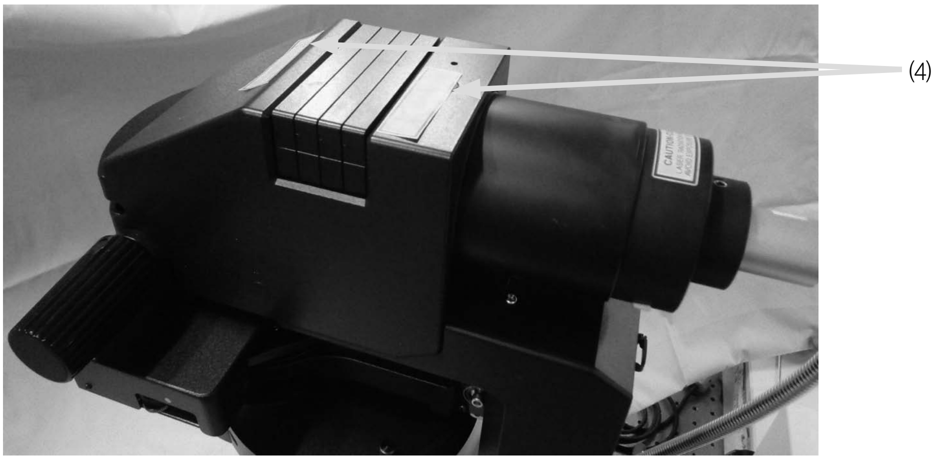
- Combination with BX61, BX61WI, IX83, and IX81



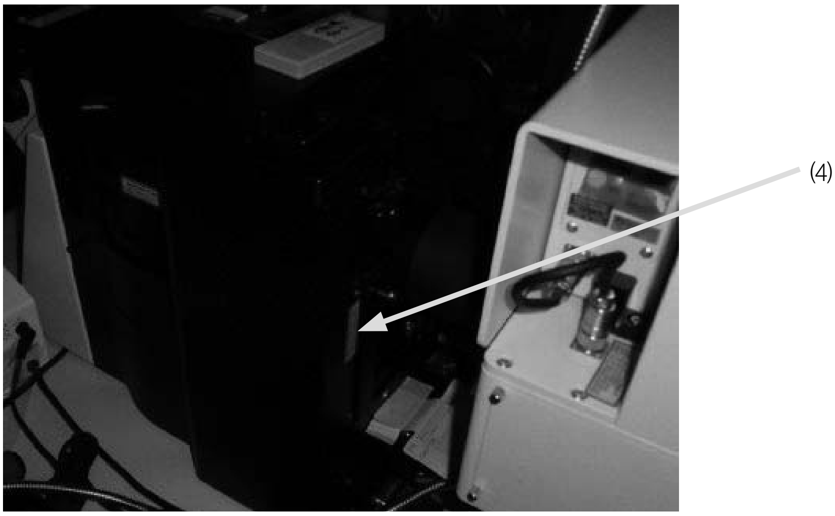
- Combination with IX83



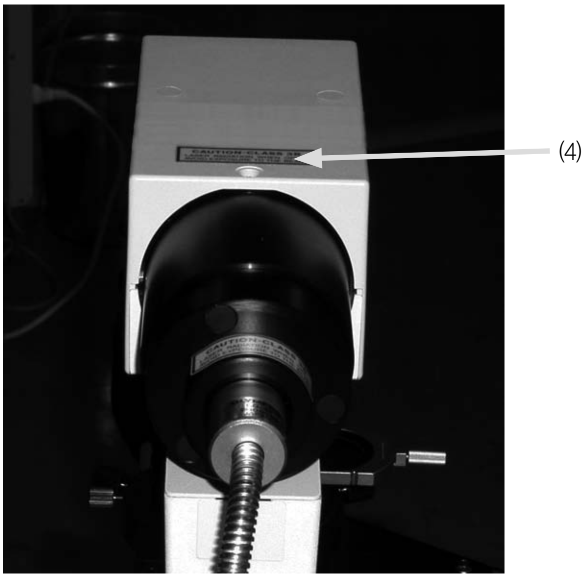
- Combination with IX83



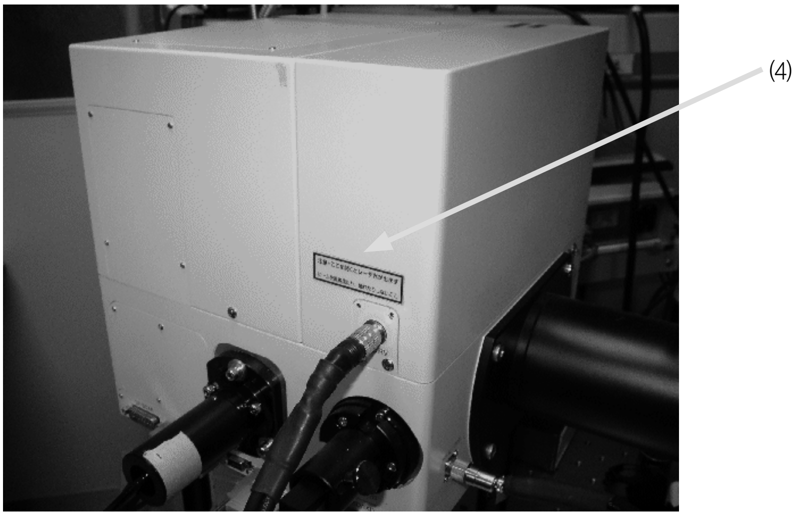
- Combination with IX83



- Combination with IX81



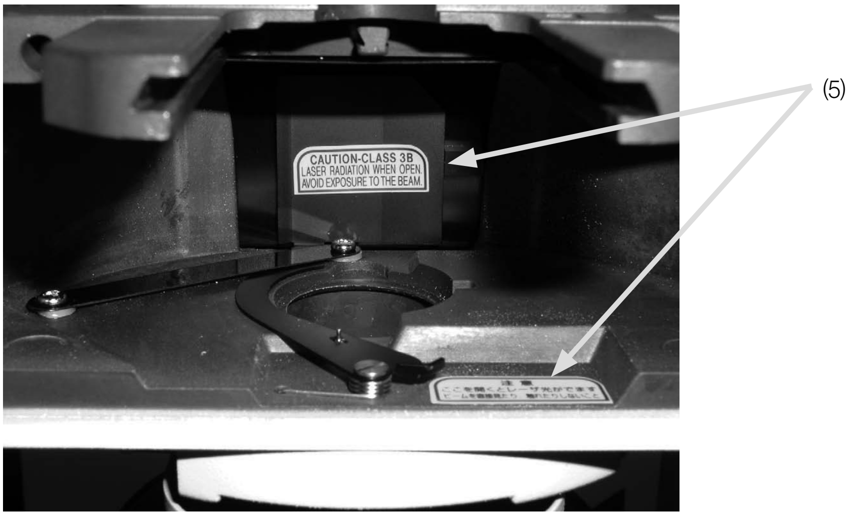
- Combination with IX83 (EVA), and IX81 (EVA)



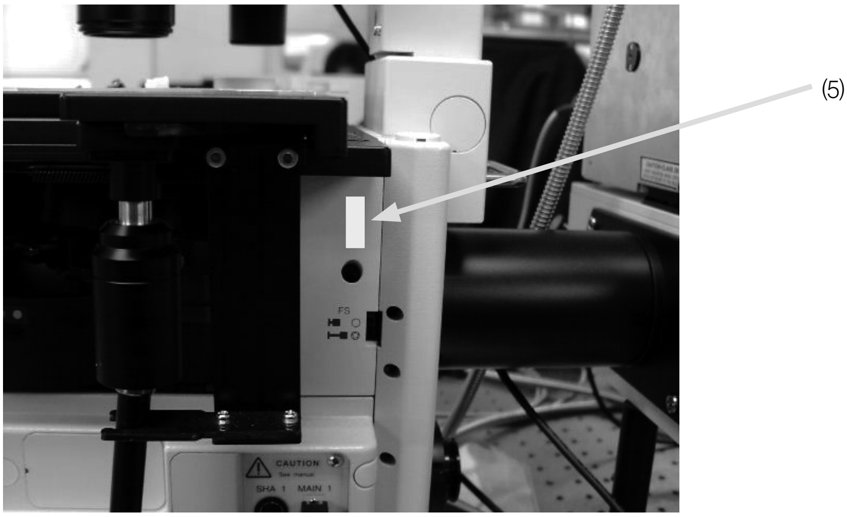
(5)

CAUTION-CLASS 3B
LASER RADIATION WHEN OPEN.
AVOID EXPOSURE TO THE BEAM.

- Combination with IBX61 and BX61WI

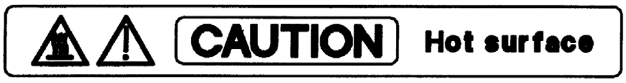

(5)
CAUTION-CLASS 3B
LASER RADIATION WHEN OPEN.
AVOID EXPOSURE TO THE BEAM.
注意
ここを開くとレーザ光がでます
ビームを直接見たり 触れたりしないこと

- Combination with IX81

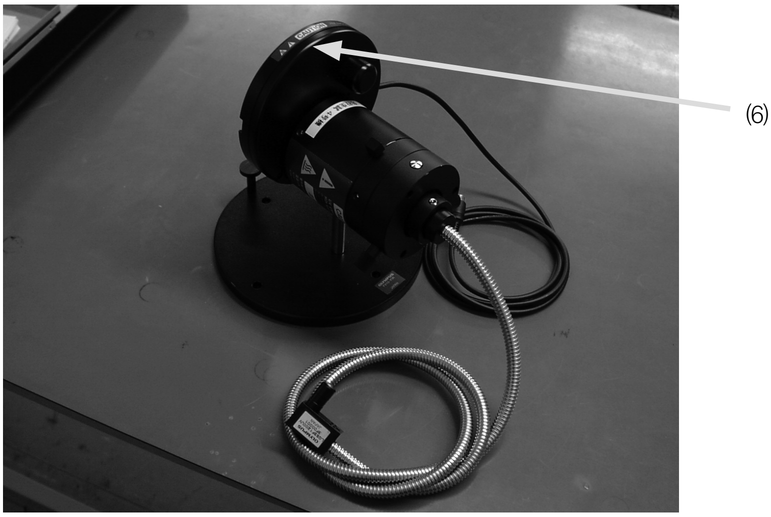

(5)
FS
CAUTION
See manual
SHA 1
MAIN 1

2-2 Other Warning Labels

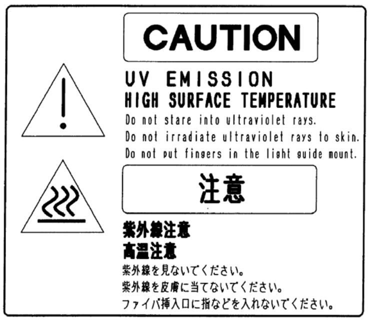
(6)


CAUTION
Hot surface

- Combination with BX61, BX61WI, IX83, and IX81



(7)



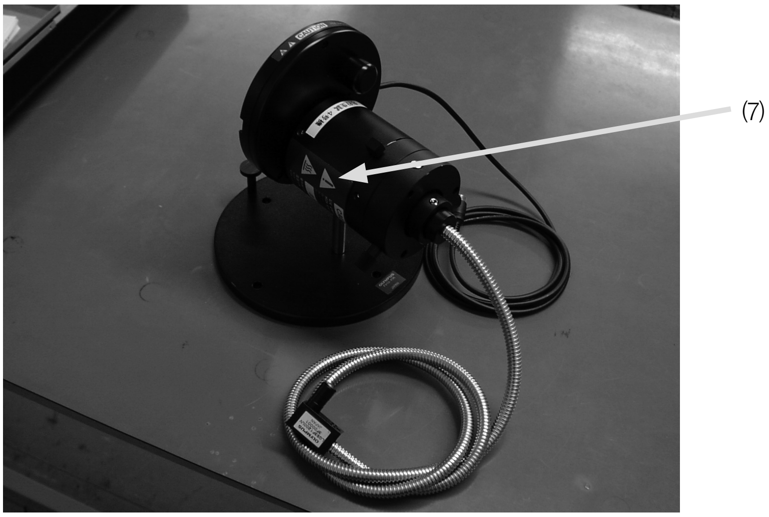
CAUTION

UV EMISSION
HIGH SURFACE TEMPERATURE
Do not stare into ultraviolet rays.
Do not irradiate ultraviolet rays to skin.
Do not put fingers in the light guide mount.

注意

紫外線注意
高温注意
紫外線を見ないでください。
紫外線を皮膚に当てないでください。
ファイバ挿入口に指などを入れないでください。

- Combination with BX61, BX61WI, IX83, and IX81



Other protective cabinet labels than those shown above are attached to other positions on the product. These labels are for Olympus service personnel to refer to.

CAUTION **For replacement of soiled or peeling warning labels, please contact your Olympus representative.**

3 CAUTION FOR SYSTEM OPERATION

3-1 Transportation of Equipment

- This system has been assembled and set up by specialist engineers and technicians. Avoid moving the system whenever possible, because this could alter the alignment of the optics. Please consult your local Olympus representative if you want to move or displace the system. If the system is moved by the user, Olympus cannot be liable, even if system trouble or damage occurs.

3-2 Treatment of System

- The laser tube used with this system should always be disposed of in accordance with local industrial waste treatment regulations. When disposal is required, please consult your local Olympus representative.

- The mercury burner used with the illumination device of this system should always be disposed of in accordance with local industrial waste treatment regulations. When disposal is required, please consult your local Olympus representative.

- In order to replace the lithium coin battery built in the controller in this system, please contact our sales office in your local area.

3-3 Handling Precautions

- This system is comprised of precision equipment. Handle it with care. Don't subject it to shock.

- Do not bend or pull the laser fiber cable with excess force or step on it, for this may lead to considerable deterioration of performance.

- Once the reflected light power is switched OFF, do not switch it ON again for at least 10 minutes. Otherwise, the life of the mercury burner may be shortened.

- Do not expose the laser head to cool air from an air conditioner, etc. Otherwise, the operation will be unstable.

- Absolutely avoid condensation of dew of the laser tube, for this will lead to explosion of the tube. When the laser tube has been located in a cold environment for a long period, do not heat the room until the laser operation has started to prevent condensation.

- Any laser should be subjected for 8 hours of continuous aging every month when it has been left unused for a month or more.
  If aging is not performed, troubles which may affect the laser service life may occur, such as instability or stoppage of laser oscillation.

- The air outlet of the laser cooling fan blows out warm air. Do not place a flammable or weak object near the heat from the air outlet. Also, to allow smooth heat radiation, provide clearance of at least 50 cm around the air outlet. (Otherwise, overheating of the laser tube may destabilize or stop the laser oscillation. The rise in the equipment's internal temperature may also cause malfunction or failure.)

- The laser power supply unit incorporates a cooling fan on the front and rear. To avoid blocking the area surrounding the cooling fan or the slits at the top and sides of the power supply unit, install the unit at a distance at least 30 cm from the surrounding walls. Also do not place objects such as papers or books on the laser power supply unit, for this may lead to overheating of the laser power supply unit or activation of the protection circuitry, causing stoppage of the laser oscillation.

- The Power Supply Unit, Laser Combiner and controller have a cooling fan on the rear. To prevent malfunction, be sure to reserve a clearance of at least 10 cm around their air outlets.

- Do not permit tools or metal fragments to get into the air outlet of the cooling fan, or other apertures. Doing so could cause failure of the units or electric shock to the user.

- Regarding Z direction move, besides mechanical limitation of microscope, a limit can separately be programmed in software within mechanical limitation.
  If a software limit is set to the position where objective lens or specimen comes close each other, for example, the damage of specimen that occurs by a contact with objective lens may be prevented.
  The software limit setting in combination use with the IX83 can be made by the touch panel controller.
  Bring [Setting] window by proceeding from [Starting-up] window - [System Setting] - [Customized] - [Focus limit setting] - [Detailed setting].
  As to the software limit setting in combination use with the BX61, BX61WI or IX81, bring ⑥ dialog box, bring [Microscope Configuration] window and enter value in [Near Limit] text box on [Focus] tab in [Microscope Configuration] dialog box.
  See Online Help – [Setting and Control of Microscope Main Unit] – [Setting Microscope Main Unit] – [About Focus tab].

- To prepare for an unexpected happening, power switch (cord) should be turned off (disconnect) easily. The system should be in a situation to be turned off(disconnect) the power switch(the power cord from inlet or wall outlet) easily anytime.

- If you want to use the simple anti-vibration table, select the one which is endurable the weight, 70kg of the equipment.

- The proper conditions to operate lasers, Multiline Ar laser, HeNe laser, and Semiconductor Laser, are at a room temperature of 18 to 28 degrees C and at humidity of 30 to 80 %.

  To extend the service life of this system, avoid using it in the following environments.
  - In a place with excessive dust or dirt
    Dust and dirt taken in the oscillator may lead to failure. Clean the system and peripherals periodically to avoid ball of dust.
  - In a place where the temperature is low or high, or where the humidity is high
    This includes, for example, a place that is near a water tap, hot-water heater, room humidifier, heater, stove and a place subject to water drops.
  - In a place where humidity changes suddenly
    When a cold room is heated quickly, moisture may condense on the equipment and could irreparably damage the optics.
  - In a place subject to direct sunlight
  - Outdoors
  - In a place where a flammable gas is produced
  - In a place with excessive vibrations
  - On an unstable surface of floor or platform

- Please carry out about exchange of consumable materials according to the handling description of each unit.

- Always use the power cord provided by Olympus. If no power cord is provided, please select the proper power cord by referring to the section ”PROPER SELECTION OF THE POWER CORD” at the end of this instruction manual. If the proper power cord is not used, the safety and EMC performance of the device can not be assured.

- Always connect the power cord correctly and ensure that the grounding terminal of the device and that of the wall outlet are properly connected. If the device is not grounded, our intended electric safety and EMC performance of the device can not be assured.

3-4 Recommendations for Use

- Combination with either an upright microscope or an inverted microscope use ordinary biological objectives. Therefore, the observed specimen should be prepared using a cover glass with thickness of 0.17 mm or a petri dish with bottom thickness of 0.17 mm. The use of a special cover glass is recommended for observations using immersion objective for LSM.

- When using this system, we recommend turning the indoor lighting low in order to reduce external disturbance light on observations. (The indoor lighting particularly affects the differential interference observation images.)

- To extend the life of laser head while it is not used for a long period, it is recommended to perform 8 hours of continuous aging at least once every month.
  Due to the properties of gas laser, the laser life becomes shorter when it is not used for a long period than when it is used continuously.

- To stabilize the laser beam output, leave the system for warm-up after turning the laser power ON, then use it. All lasers should be warmed up for more than 30 minutes before use.

3-5 Care and Storage

- When the microscope is not in use, be sure to set the main switch to "O" (OFF). After checking that the lamp housing has been cooled down, place the dust-prevention cover on the microscope for storage.

- Never leave stains or fingerprints on the lenses and filters. Should a glass component be stained, simply blow dirty away using a commercially available blower and wipe gently using a piece of cleaning paper (or clean gauze).
  If a lens is stained with fingerprints or oil smudges, wipe it gauze slightly moistened with commercially available absolute alcohol.

CAUTION **Since the absolute alcohol is highly flammable, it must be handled carefully.**
Be sure to keep it away from open flames or potential sources of electrical sparks – for example, electrical equipment that is being switched on or off.
Also remember to always use it only in a well-ventilated room.

- To clean a part, wipe with a soft cloth moistened with a little drop of diluted neutral detergent. Do not use organic solvents that will deteriorate the surface coating and plastic parts.

3-6 Caution

If the equipment is used in a manner not specified in this manual, the safety of the user may be imperiled. In addition, the equipment may also be damaged. Always operate the equipment following this user's manual.

3-7 Controller and Software

CAUTION **The controller data may be destroyed by accident. Be sure to back up the controller data as required.**

- Olympus cannot assume liabilities for any auxiliary damage, including compensation for loss of controller data, resulting from use of or inability to use this product.

- The controller used with this system has already been set up with Microsoft Windows.
  This operating system should be backed up and the backup retained carefully by the user. (Olympus does not provide backup support.)
  For details on the controller and Microsoft Windows, also refer to the manuals provided with them.

- Quality assurance for this system is provided at ex-factory basis.
  Be advised that any operating failure or disordered function due to Environment Setting Change (BIOS change) or installation of other software by user is not covered by the warranty.

- When the remaining available hard disk space becomes small, the data processing rate may drop drastically or errors may tend to occur. To prevent this, delete unnecessary data files frequently. For the data file deletion procedure, refer to the manuals provided with Microsoft Windows.
  Free space of hard disk should be at least of 1Gbyte.

- Disable the screen saver (by setting it to None) before use.
  With this software, the screen saver has already been set to "None" at the factory.

- Never delete the folder or modify its name, which created in the hard disk of the controller in advance,
  or the software would not be starting up.

- Never activate other softwares while this software is running, or it might cause malfunction of this software.

- Please do not connect the USB unit which is not provided by us to controller to be used. Connecting the USB unit which is not provided by us may cause the malfunction.

- When connecting the controller to the network, install the antivirus software. However, there may be a case that the system does not work properly when the antivirus software is installed.

- If the antivirus software has been installed in controller to be used, the unexpected problem, e.g. missing image, may occur. Prior to using this system, disconnect controller network connection to stop the antivirus software.

- This system is equipped with the protection function to reduce the drastic deterioration of GaAsP-PMT attached to FV12-HSD. If the excessive light enters GaAsP-PMT, [HV] becomes [OV] and the warning tone beeps.

- After the protection function is enabled, actions during scanning is different from actions during non-scanning. In case of scanning, [HV] returns to the original value after one second and the warning tone stops beeping.

- In case of non-scanning, the warning tone also stops beeping after one second, but [HV] stays as [0V]. In additon, on the FV10-ASW software, the information that the protection function is enabled is displayed and HSD1 and HSD2 of PMT are unchecked at the same time.

SPECIFICATIONS

On This Volume

This volume describes the specifications of the FLUOVIEW FV1200 system.
Please read this volume so that you can understand the system before use.

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1 SPECIFICATIONS

1-1 Specifications of Units

Unit		Specifications
Laser Combiner FV10-MCOMB/S-2 Single port type FV10-MCOMB/D-2 Dual port type	Optical fiber cable	Polarized preserved fiber cable (length 3 m) (FV10-FURBB) FV10-MCOMB/S-2: 1 cable, FV10-MCOMB/D-2: 2 cables
	Dimensions(mm)	514~640(W) x 364~504(D) x 236(H)
	Weight	MAX : 40kg
	Intensity adjustment	AOTF Operation continuous change 0.1 to 100%, 0.1%step
	Operating Mode	Normal : Laser shut-off during beam flying back ROI : Intensity can be defined for each region Laser line can be selected for each region
Gas laser	Multiline Ar laser FV5-LAMAR-2	22mW output, linear polarization, continuous wave forced air-cooled Ar ion laser (457.9nm, 488nm, 514.5nm) Beam divergence: 1 mrad Power consumption: 90~264V, 14A(max.)
	Multiline Ar laser FV5-LAMAR	40mW output, linear polarization, continuous wave forced air-cooled Ar ion laser (458nm, 488nm, 515nm) Beam divergence: 0.95 mrad Power consumption: 100~240V, 20A(max.)
	HeNe (G) laser FV5-LAHEG-2	1 mW output, linear polarization, continuous wave natural air-cooled Green HeNe laser (543.5 nm) Beam divergence: 0.9 mrad Power consumption:100~120V, 0.6A(max.)
	HeNe (G) laser FV10-LAHEG230-2	1 mW output, linear polarization, continuous wave natural air-cooled Green HeNe laser (543.5 nm) Beam divergence: 0.9 mrad Power consumption:180~264V, 0.3A(max.)
Semiconductor Laser	LD405	Head output 56mW, liner polarization, 405nm, continuous wave Beam divergence: 0.6 mrad With temperature control circuit using Peltier device
	LD440	Head output 29mW, liner polarization, 440nm, continuous wave Beam divergence: 0.6 mrad With temperature control circuit using Peltier device
	LD473	Head output 17mW, liner polarization, 473nm, continuous wave Beam divergence: 0.6 mrad With temperature control circuit using Peltier device
	LD559	Head output 100mW, liner polarization, 559nm, continuous wave Beam divergence: 1.3 mrad (Max.), 1.1 mrad (Typical) With temperature control circuit using Peltier device Power consumption: AC100~120 / 220~240V 30W(max) 50/60Hz
	LD635	Head output 23mW, liner polarization, 635nm, continuous wave Beam divergence: 0.6 mrad With temperature control circuit using Peltier device

Unit		Specifications
Scan unit FV12-SU Microscope Adapter FV10-SUBX FV12-SUIX FV10-SUWI	Basic Construction	The Scan Unit (FV10- SU) is adapted to the back of microscope using Microscope adapter (FV10-SUBX, FV12-SUIX, FV10-SUWI) Prepared 3 ports for laser introduction (Visible, UV (option), IR(more than 700nm)) Detector Choice for SU : 3CH HQ ion-coating Filter Type 2CH Spectrometer + 1CH Filter Type Optional SU port available for twin scanner or 2CH non-confocal detector
	Scanning Device	Point optical scanning system with 2 galvano-meter mirrors
	Scanning mode XY scanning Sub mode: ZoomIN, Clip Multi-dimensions : T, Z, Ramda Free factor combination Line Scan Point Scan	 Pixels : 64*64 ~ 4096*4096 Aspect ratio : 1:1, 4:3, or Arbitrary ratio Scanning Speed (Pixel duration) : 2us ~ 200us , 9 steps Dimension : Time, Z, Ramda (combine freely) Line Type : Straight line (incl. Rotation), Free line Number of lines : 1 – 32500 Lines
	Field of view (FN)	18
	Zoom factor	1- 50 X, 0.5x step
	Optical Path Switching	Motorized Path Switching : LSM / FL Reflected Light Illuminator / External Optional Port
	Filter Cube Turret	Mount conventional cubes on 4 positions out of 6 on the turret. One position is dedicated to the LSM path. IX81 : Motorized 6 position BX61 : Motorized 6 position BX61WI : Manual 6 position
	Dimensions (mm)	324(W) x 487.5(D) x 110(H) (FV12-SUIX) 324 (W) x 603.7(D) x 110(H) (FV10-SUBX) 324 (W) x 603.7 (D) x 110(H) (FV10-SUWI)
	Weight FV12-SU	10.00kg (with FV12-SUIX) 12.90kg (with FV10-SUBX) 11.18kg (with FV10-SUWI)

Unit		Specifications
Fluorescence Detector Unit		
Filter type fluorescent Detector FV10-FD	Detector	Hamamatsu Photomultiplier R7862 (1/2inch R6357 high sensitivity type) 3ch standard / 1ch option
	Pinhole	Single Pinhole Pinhole size : Φ50-800um changeable 5um step
	Wavelength Selection	- SDM + HQ ion coating Barrier Filter 3CHs (standard) - 6 position turret for SDM, Barrier Filter on each CH
	Dimensions (mm)	270 (W) x 162 (D) x 144 (H)
	Weight	6.6kg
Spectral fluorescent detector FV10-SPD	Spectra type Detector	CH1 and CH2 has individual optical gratings and slits. The grating angle defines the wavelength of the detecting fluorescence. The wavelength width are selected by the slit on each channel .
	Spectra channel	Number of Channels : 2 Resolution : ‹ 2nm Setting resolution : 1nm Switching speed : 1ms / 100nm Wavelength Width : 1-100nm Channel separation : SDM (6 position)
	Filter Channel(s)	Number of Channel(s) : 1 or 2 SDM + HQ ion coating Barrier Filter (6 position each)
	Detector	Hamamatsu Photomultiplier R7862 (1/2inch R6357 high sensitivity type) 3ch standard / 1ch option
	Pinhole	Single Pinhole Pinhole size : Φ50-300um changeable 5um step
	Wavelength Selection	Spectra type - SDM + Grating type spectrometer : CH1, CH2 - SDM + HQ ion coating Barrier Filter : CH3 (CH4)
	Dimensions (mm)	270 (W) x 162 (D) x 144 (H)
	Weight	6.6kg

Unit		Specifications
Power Supply Unit FV10-PSU-2	Dimensions (mm)	180 (W) x 328 (D) x 424(H)
	Power consumption	100~120 / 220~240V AC 4.0 / 2.0A(max.) 50/60Hz
	Weight	7.5kg
Power Supply Unit for the Laser combiner FV10-MCPSU	Dimensions (mm)	95 (W) x 210 (D) x 297 (H)
	Power consumption	100~120/220~240V AC 2 / 1A(max.) 50/60Hz
	Weight	4.3kg
Microscope [Upright type]	Models	BX61, BX61WI, BX62, BX62WI,
	Objectives	UIS series objectives
[Inverted type]	Models	IX83, IX81
	Objectives	UIS series objectives
	Z Drive System	Motorized Focusing Module (Microscope frame built-in) Moving step : 0.01 um During Z series scanning, the Focusing Module is directly controlled by FV10-PSU for faster frame rate.
Transmitted Light Detector FV10-TD		via the Bandle fiber onto the transmitted light illuminator part with the microscope frame. Motorized Switching for Transmitted Light Detector / Transmitted Light Illumination
	Detector	Hamamatsu Photomultiplier R7400
	Lamp house	Halogen LH, U-LH100-3, mounted on the Detector Unit
	Dimensions (mm)	210(W) x 170(D) x 124(H)
	Weight	3.5kg
Epi Fiber Illumination Unit FV10-FIR		Fiber connection from Lamp house to SU, Provided with motorized shutter Motorized switching between LSM path and Illuminator path.
	Dimensions (mm)	218.4(W) x 180(D) x 210(H)
	Weight	4.48kg
Optional high sensitivity descan detector FV12-HSD	Wavelength Selection	Filter cube (SDM + Barrier Filter)
	Detector	Hamamatsu Photomultiplier H10771
	Dimensions (mm)	230(W) x 215(D) x 191(H)
	Weight	5.4kg
Fiber Port for Fluorescent Detection FV10-FPT		Fiber connector type : FC Fiber core diameter : 100-125um NA : 0.2
Additional 4th channel fluorescent detector FV10-OP4CH	Wavelength Selection	SDM + HQ ion coating Barrier Filter
	Detector	Hamamatsu Photomultiplier R7400
	Dimensions (mm)	94(W) x 99.5(D) x 144(H)
	Weight	2kg

Item	Specifications
Laser radiation from the system	- Wavelength: 400 to 700 nm - Maximum power: 50 mW - Continuous wave - Beam divergence: 0.15 to 1.2 rad (through the objective lens)
Operating environment	- Indoor use. - Altitude : Maximum 2000m - Ambient temperature : 18°C to 28°C (Fluctuation ±1°C) Maximum relative humidity 75% (No dew) - Main supply voltage fluctuations not to exceed ±10% of the nominal voltage. - Pollution Degree 2 (in accordance with IEC 60664-1) - Installation / Over voltage Category Ⅱ (in accordance with IEC 60664-1)

CE Compliance

This device complies with the requirements of both directive 2004/108/EC concerning electromagnetic compatibility and directive 2006/95/EC concerning low voltage. The CE marking indicates compliance with the above directives.

Safety and EMC Precautions

1. Use only power cord which OLYMPUS specifies. Otherwise the safety and EMC performance of the product can not be assured.
2. Be sure to ground the product. Otherwise our intended electric safety and EMC performance of the product can not be assured.

The directives are applied based on the conformance to the requirements of technical standards.
The standards below are used so as to verify the compliance with the directives.

Safety
EN61010-1(2001:2nd Edition): Safety requirements for electrical equipment for measurement, control and laboratory use.

Electromagnetic Compatibility
EN61326-1:2006: Electrical equipment for measurement, control and laboratory use EMC requirements.

EN61326-1 defines two categories according to the location for use.
Class A: Equipment suitable for use in establishments other than domestic, and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.
Class B: Equipment for use in domestic establishments, and in establishments directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

This system is applied Class A. Some interference may occur if this system is used in domestic location.

The system configuration of the Class A is as follows:

1-2 Power Consumption

Some unit may require a large amount of power consumption as shown below. When using each unit, verify the capacity of power outlet to be used and then, use it. The system configuration may be changed without notice.

- FV1200 Power Supply Rated

Unit	Name	Voltage Rate (AC)	Frequency	AC100V Maximum current	AC200V Maximum current
BX-UCB/ IX-UCB-2	Universal Control Unit	100-120V/220-240V Auto selection	50/60Hz Auto selection	3.5A	1.5A
IX3-CBH	Universal Control Unit	100-120V/220-240V Auto selection	50/60Hz Auto selection	4.6A	2.8A
U-TPC	Touch Panel Controller	100-240V Continuous change	50/60Hz Auto selection	1.7A	
FV10-PSU-2	Power Supply unit for FV1200	100-120V/220-240V Auto selection	50/60Hz Auto selection	4A	2A
FV10-MCPSU	Power Supply for MultiCombiner	100-120V/220-240V Auto selection	50/60Hz Auto selection	2A	1A
FV5-LDPSU	Laser Diode Power Supply Unit	100-120V/220-240V Auto selection	50/60Hz Auto selection	0.9A	0.5A
FV5-LAMAR-2	Multi Argon Laser Unit	100-120V/220-240V Auto selection	50/60Hz Auto selection	15A	8A
FV5-LAHEG-2	He-Ne Green Laser Unit	100-120V	50/60Hz Auto selection	0.6A	-
FV10-LAHEG230-2	He-Ne Green Laser Unit	100-240V	50/60Hz Auto selection	-	0.3A
FV10-LD559	LD559 Laser Unit	100-120V/220-240V Auto selection	50/60Hz Auto selection	0.3A	0.15A
FV12-CTR-SET	Controller	100-240V Continuous change	50/60Hz Auto selection	6A	3A
FV10-LCD19	Monitor	100-120V/220-240V Auto selection	50/60Hz Auto selection	0.8A	0.4A
FV10-LCD30	Monitor	100-240V Continuous change	50/60Hz Auto selection	1.85A	0.9A
U-RFL-T	Power Supply Unit for Mercury Lump	100-120V/220-240V Auto selection	50/60Hz Auto selection	1.6A	0.8A
U-HGLGPS	Power Supply Unit for Mercury Lump	100-240V Continuous change	50/60Hz Auto selection	2.0A	

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OLYMPUS®

User's Manual
[Operation/Maintenance Manual]

FLUOVIEW
FV1200

BIOLOGICAL CONFOCAL LASER SCANNING MICROSCOPE

FV10-ASW

Notice

Thank you for your purchase of Olympus microscope at this time.

Retain this manual in an easily accessible place near a system for future reference.

AX8266

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IMPORTANT

CAUTION

FV1200 is a CLASS 3B laser product.
The procedures for using this system are classified as follows:

- Service
  "Service" means any adjustment or repair performed by service personnels who are provided the service training following to the service manual for this system.
  The performance has influence on the feature of this system, and there is a risk which unintended CLASS 3B laser light is emitted.
- Maintenance
  "Maintenance" means adjustment or other procedures performed by customers to maintain that this system functions properly.
- Operation
  "Operation" means all performance described in the user's manuals in this system.
  CLASS 3B laser light is only emitted from the objective lens during the actual execution.

The User's Manuals of this system consist of the following:
In order to maintain the full performance of this system and ensure your safety, be sure to read these user's manuals and the operating instructions for the laser unit and light source unit before use.

	User's Manual Sets	
	FV1200MPE	FV1200
FV1200MPE / FV1200 User's Manual [Laser Safety Guide]	○	○
FV1200MPE User's Manual [Safety Manual]	○	
FV1200 User's Manual [Safety Manual]	○	○
FV1200MPE User's Manual [Operation / Maintenance Manual]	○	
FV1200 User's Manual [Operation / Maintenance Manual]	○	○
FV1200 FV10-ASW User's Manual [Quick Start]	○	○

Also, we have prepared one service manual for this system as below. Technical personnels who perform the service are required to take the service training.

- FV1200MPE / FV1200 Service Manual

Reproduction, copying or duplication of a part or all of this software and manual is prohibited.

For customers using FV1000MPE or FV1000:
This manual contains the instructions for FV1200 and FV-10ASW (Ver.4.0 and later version).
If the instructions for FV1000MPE / FV1000 and (former version than Ver.4.0) are not covered by this manual,refer to the manual for FV1000MPE / FV1000 on your hand.

Registered Trademarks

Microsoft, Microsoft Windows, Excel for Windows are registered trademarks of Microsoft Corporation.
Other brand names and product names are trademarks or registered trademarks of their respective owners.

Caution

If the system is used in a manner not specified by this manual, the safety of the user may be imperiled. In addition, the system may also be damaged. Always use the system as outlined in this instruction manual.

The following symbols are used to set off text in this instruction manual.

CAUTION : Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury or damage to the equipment or other property. It may also be used to alert against unsafe practices.

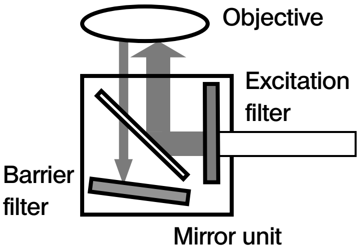
◎ : Indicates commentary (for ease of operation and maintenance).

Precautions and Notes for Glare Prevention

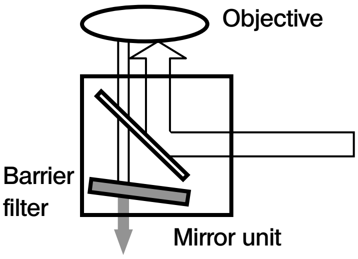
══When excitation filter is removed from the fluorescence mirror unit══

When the excitation filter is removed from the fluorescence mirror unit and attached on the excitation filter slider or excitation filter wheel on the side of the white lamp, **very glaring light (*1) may enter the eyepieces** in the following cases.

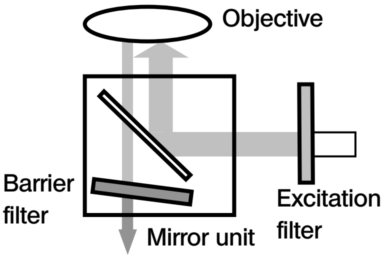
① When the illumination light from the white light lamp (*2) is input **without passing through an excitation filter.**
② When the illumination light from the white light lamp is input through **an excitation filter that does not match** the mirror unit type.



Ordinary mirror unit
Light transmitted through the excitation filter is blocked completely by the barrier filter.



Case of ① above
Among the white light, only the light transmitted through the barrier filter enters the observation light path.



Case of ② above
Light transmitted through the excitation filter is sometimes blocked incompletely by the barrier filter.

To avoid this problem, be sure to observe the following points.

- Before switching the mirror unit and excitation filter, be sure to close the shutter of the mirror unit turret.
- Before opening the shutter of the mirror unit turret, be sure to check the position index of the mirror unit to confirm that it matches the type of the excitation filter engaged in the illumination light path.

(*1) The light will not injure your eyes even if it enters your eyes. However, be sure to stop observation through the eyepieces, and engage the mirror unit and the excitation filter of appropriate combination in the light path to restart observation.
(*2) The white light lamp refers to all lamps for reflected light illumination including mercury and xenon burners.

CAUTION :Never use the above-mentioned mirror units ① and ② for the LSM observation.

I. SYSTEM OVERVIEW

On This Volume

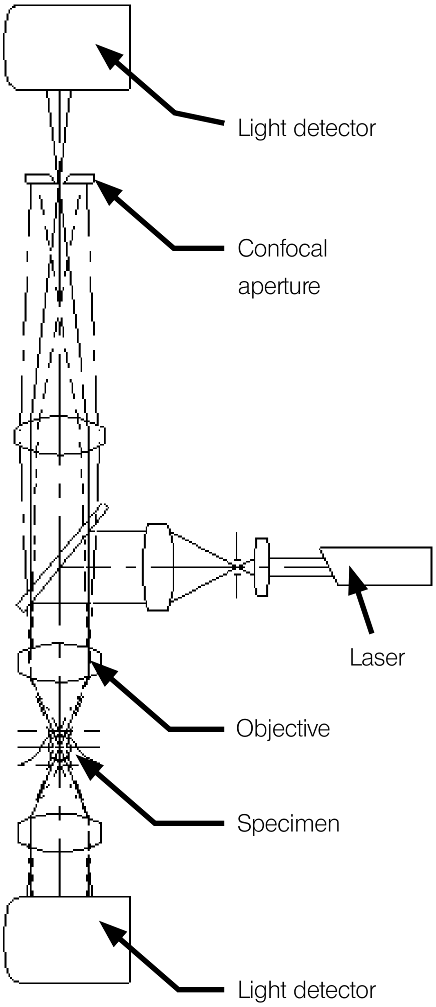
This volume describes the overview of the FLUOVIEW FV1200 system.
Please read this volume so that you can understand the system before use.

1 System Overview

OLYMPUS FV1200 is a confocal laser scanning biological microscope system featuring improved basic performances (sensor system, scanning system and illumination system performances) by considering the “live cell observations”, with which long hours of stable measurement of weak fluorescence is required.
This microscope is equipped with 3 fluorescence channels, 3 lasers and AOTF to meet various applications in a wide range of advanced research fields.

1-1 Principles

A laser scanning microscope converges the laser beam into a small spot using an objective and scans the specimen in the X-Y direction using the laser beam. The microscope then captures the fluorescent light and reflected light from the specimen using light detectors and outputs the specimen image on an image monitor.
As shown in this figure, the confocal optics incorporates a confocal aperture on the optically conjugate position (confocal plane) with the focus position to eliminate light from other part than the focus position. This causes the extraneous light to be viewed as darkness in the observation image, it is possible to slice optically a tissue specimen that has thickness.
On the other hand, an ordinary optical microscope, the light from other part than the focus position is overlapped with the imaging light of the focus position so the image is blurred in overall.
The laser beam that has transmitted through the specimen is detected by the transmitted light detector and provides the transmitted image, which is not a confocal image.
However, when the fluorescence images of the transmitted and confocal images are combined, it is possible to obtain very important information on the specimen.



1-2 Features of the FV1200

1. The photon counting mode is provided to improve the sensitivity and S/N and to enable quantitative optical intensity measurement. Photon counting makes possible long hours of quantitative observation by completely eliminating analog-derived drift. The dynamic range in which photon counting is possible is expanded using a newly designed wideband head amplifier and processing circuitry.

2. High-speed imaging at 8 frames per sec. is made possible by fast galvano mirror. In addition, high-speed image acquisition is possible without stopping the Z-series motors used in the XYZ and XZ observations.

3. During long hours of time-lapse observation, a stable supply of excitation light is made possible thanks to the feedback control of the intensity of each laser. Together with the photon counting function, this function ensures the stability and quantitative nature of long-hour observations.

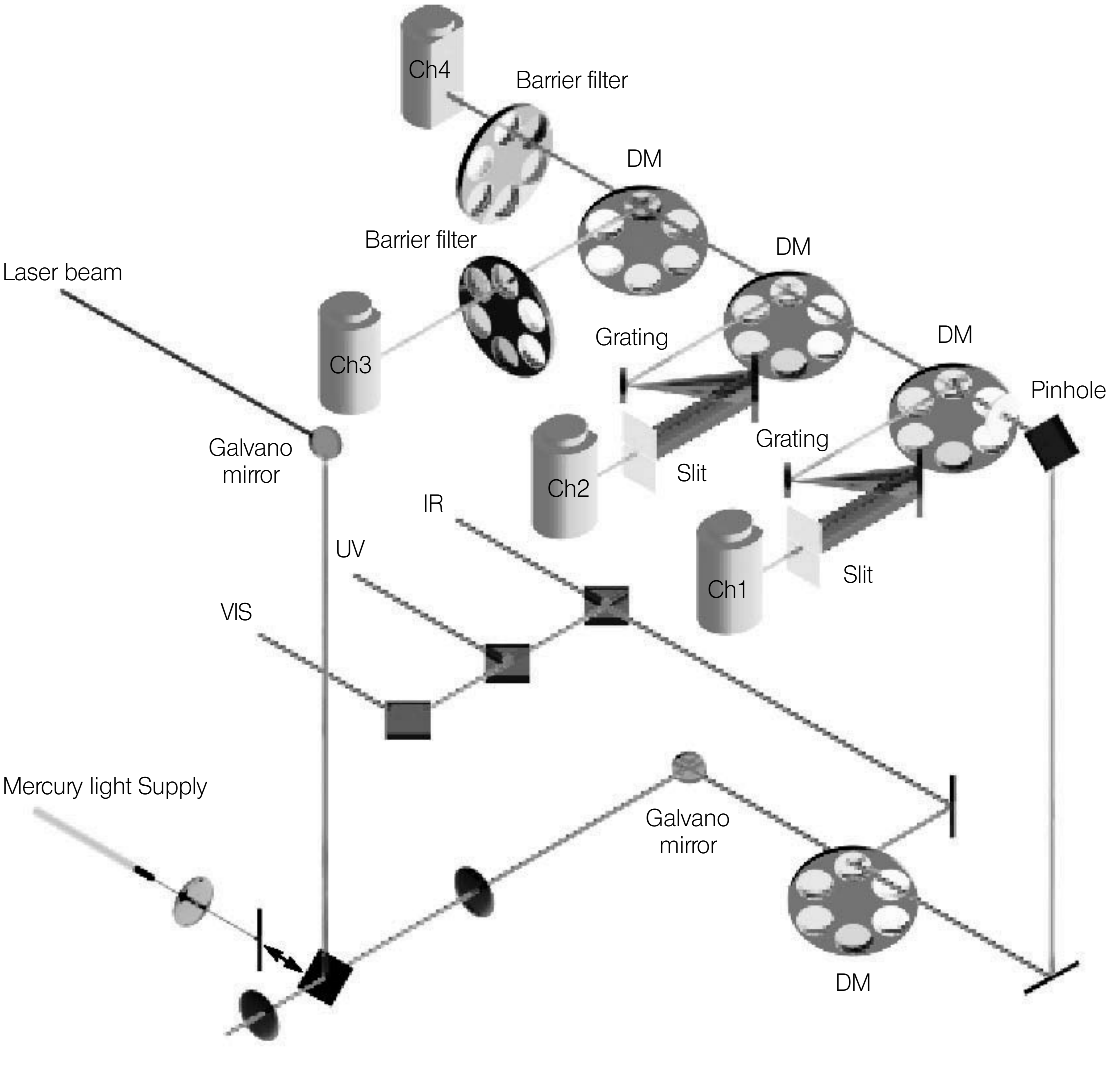
4. Three fluorescence channels, three lasers and AOTF are provided as standard to meet a large variety of applications.

5. With a fully-motorized scan unit and motorized microscope, the entire system is motorized so the scanning conditions including those of the optics can be saved and reproduced.

6. When an extension laser irradiation unit is used for photon activation aiming at causing discoloration, optical simulation or uncaging of the specimen, a system optimized for cell function analysis experiments can be built.

7. When the system incorporates the spectral detector unit that is composed of a 2-channel spectral detector and 1-channel filter, it is possible to set the detection conditions more flexibly, acquire the fluorescence spectral data and use the fluorescence isolation function.

1-3 Optical Path Diagram

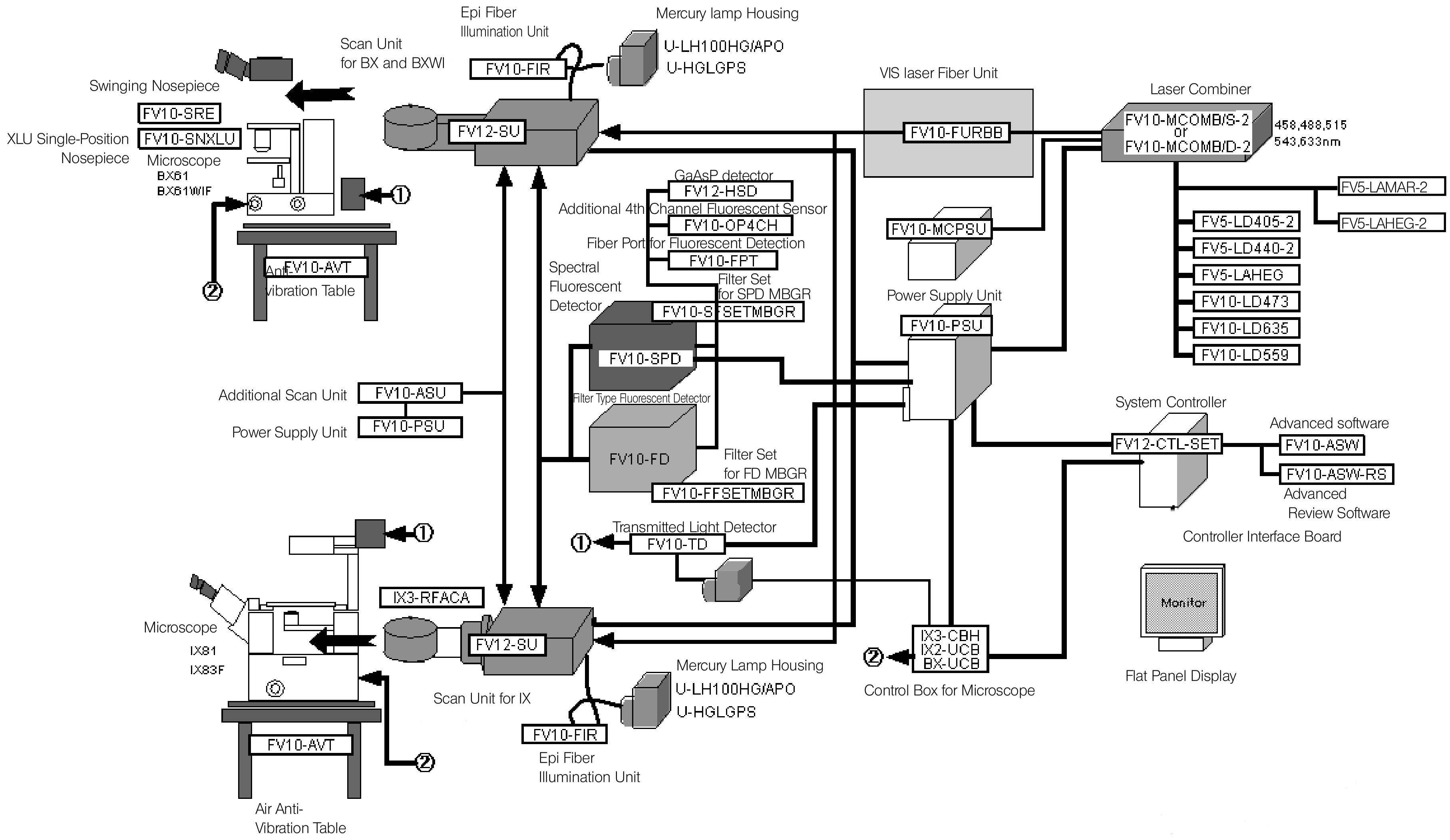

Ch4
Barrier filter
DM
Barrier filter
DM
Laser beam
Grating
DM
Ch3
Pinhole
Grating
Galvano mirror
Slit
Ch2
IR
UV
Ch1
Slit
VIS
Mercury light Supply
Galvano mirror
DM

◎ This Optical Path Diagram present the system configuration where Additional 4th Channel Fluorescent sensor (FV10-OP4CH) is attached with Spectral Fluorescent Detector (FV10-SPD).

- When the Filter Type Fluorescent Detector (FV10-FD) is attached, instead of the Spectral Fluorescent Detector (FV10-SPD), the Grating and Slit engaged in the Ch1 and Ch2 light path is changed into the Barrier filter in this Diagram.
- When the GaAsP detector (FV12-HSD) is attached, instead of the Additional 4th Channel Fluorescent sensor (FV10-OP4CH), the Ch4 is changed into the HSD1 and HSD2 in this Diagram.

1-4 System Configuration

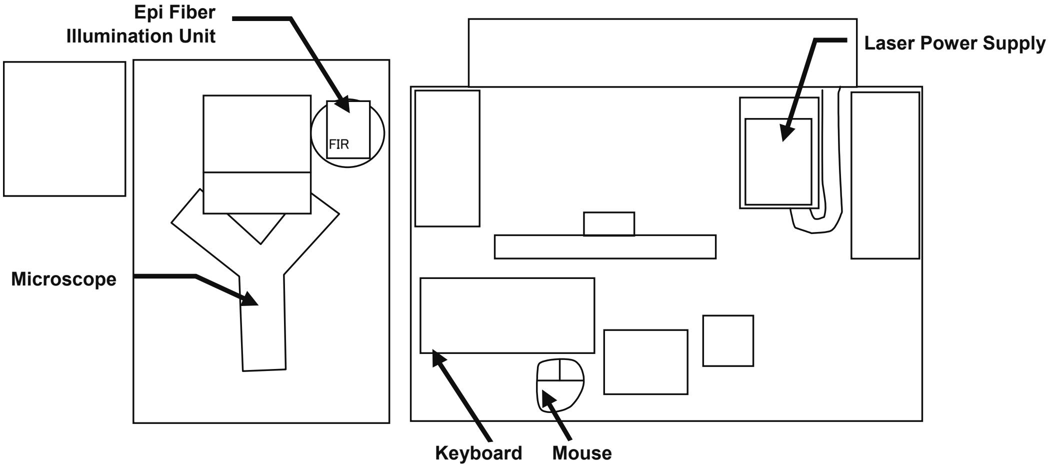
1 System Diagram


Epi Fiber
Illumination Unit
FV10-FIR
Mercury lamp Housing
U-LH100HG/APO
U-HGLGPS
Scan Unit
for BX and BXWI
Swinging Nosepiece
FV10-SRE
XLU Single-Position Nosepiece
FV10-SNXLU
Microscope
BX61
BX61WIF
FV12-SU
FV10-AVT
Anti-vibration Table
①
②
VIS laser Fiber Unit
FV10-FURBB
Laser Combiner
FV10-MCOMB/S-2
or
FV10-MCOMB/D-2
458,488,515
543,633nm
FV5-LAMAR-2
FV5-LAHEG-2
FV5-LD405-2
FV5-LD440-2
FV5-LAHEG
FV10-LD473
FV10-LD635
FV10-LD559
GaAsP detector
FV12-HSD
Additional 4th Channel Fluorescent Sensor
FV10-OP4CH
Fiber Port for Fluorescent Detection
FV10-FPT
Spectral
Fluorescent
Detector
Filter Set
for SPD MBGR
FV10-SFSETMBGR
FV10-SPD
Filter Type Fluorescent Detector
FV10-FD
Filter Set
for FD MBGR
FV10-FFSETMBGR
FV10-MCPSU
Power Supply Unit
FV10-PSU
Additional Scan Unit
FV10-ASU
Power Supply Unit
FV10-PSU
System Controller
FV12-CTL-SET
Advanced software
FV10-ASW
FV10-ASW-RS
Advanced
Review Software
Controller Interface Board
Monitor
Flat Panel Display
Transmitted Light Detector
FV10-TD
IX3-CBH
IX2-UCB
BX-UCB
Control Box for Microscope
IX3-RFACA
Microscope
IX81
IX83F
FV12-SU
Scan Unit for IX
Mercury Lamp Housing
U-LH100HG/APO
U-HGLGPS
FV10-FIR
Epi Fiber
Illumination Unit
FV10-AVT
Air Anti-
Vibration Table

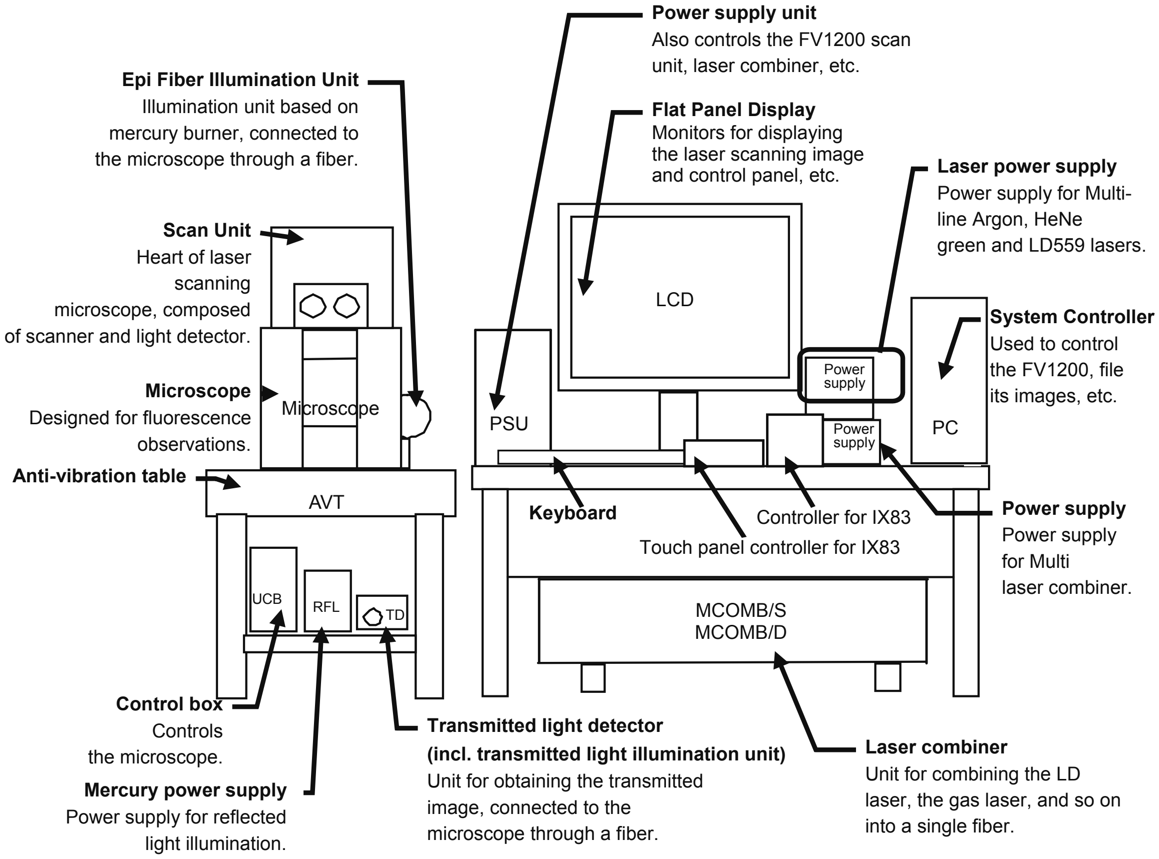
◎ When additional microscope units are required to the system, Olympus service engineer will perform the installation.

2 System Appearance and Functions

The applicable microscopes are the BX61/62TRF, BX61WIF, IX83P2ZF and IX81F.


Epi Fiber Illumination Unit
Laser Power Supply
FIR
Microscope
Keyboard
Mouse

Top View


Microscope
LCD
Power supply
PSU
PC
Power supply
AVT
UCB
RFL
TD
MCOMB/S
MCOMB/D
Keyboard
Controller for IX83
Touch panel controller for IX83

Power supply unit
Also controls the FV1200 scan unit, laser combiner, etc.

Epi Fiber Illumination Unit
Illumination unit based on mercury burner, connected to the microscope through a fiber.

Flat Panel Display
Monitors for displaying the laser scanning image and control panel, etc.

Laser power supply
Power supply for Multi-line Argon, HeNe green and LD559 lasers.

Scan Unit
Heart of laser scanning microscope, composed of scanner and light detector.

System Controller
Used to control the FV1200, file its images, etc.

Microscope
Designed for fluorescence observations.

Anti-vibration table

Power supply
Power supply for Multi laser combiner.

Control box
Controls the microscope.

Transmitted light detector (incl. transmitted light illumination unit)
Unit for obtaining the transmitted image, connected to the microscope through a fiber.

Laser combiner
Unit for combining the LD laser, the gas laser, and so on into a single fiber.

Mercury power supply
Power supply for reflected light illumination.

Front View

II. PREPARATION For OPERATION

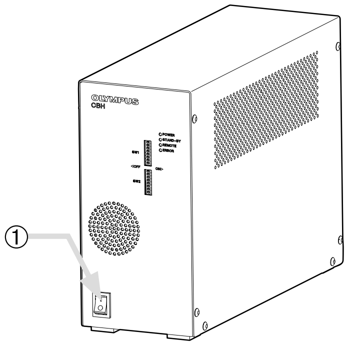
On This Volume

This volume describes the methods for preparation for operation of the FLUOVIEW FV1200 system.
After completing the preparations, activate the software and start observation by controlling the display on the monitor screen.
Please read this volume so that you can understand the system before use.

1 Preparation for Operation

1-1 Turning the Power On

IX3-CBH
Main switch



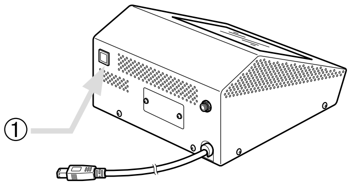
1.Set the power switches of the controller and the monitor to I (ON).

2.Set the power switches of the units of microscope to I (ON).
When using the IX83P2ZF microscope
◎ Set the main switches of the units to O (OFF) in the following order:
1) Main switch ① of the IX3-CBH
2) Main switch ① of the touch panel controller

3. Set the power switches of the following FV1200 units to I (ON).

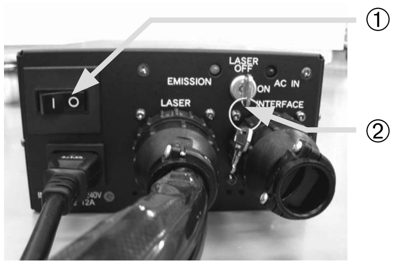
- Power Supply Unit FV10-PSU
- Mercury Burner Power Supply Unit
- Microscope Control Box BX-UCB , IX3-CBH or IX2-UCB

Touch panel controller
Main switch



4. Set the power switches of the lasers to I (ON).

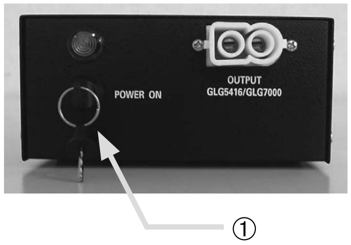
FV5-LAMAR-2 Power Supply



4.1 Multi-line Argon laser: FV5-LAMAR-2

- Set the power switch ① to ON. (This starts the fan of the laser.)
- Turn the key ② to the ON position.
  It takes a few tens of seconds after the key ② is set to ON till the laser oscillation begins.

FV5-LAHEG-2 Power Supply

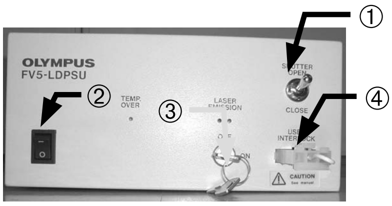


4.2 Helium-Neon Green Laser: FV5-LAHEG-2 or FV10-LAHEG230-2

- Turn the key ① to the I (ON) position.
  It takes a few tens of seconds after the key is set to ON till the laser oscillation begins.

CAUTION **To ensure stable laser light output, it is recommended to warm up the laser power supply after turning it on. The warm-up period should be 10 minutes or more when using the Argon laser power supply and 30 minutes or more when using the Helium-Neon Green or Red laser power supply.**

4.4 LD405/440 laser: FV10-LD405/440

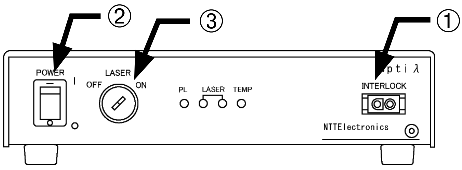


LD405/440 laser power supply

- Make sure that the provided shorting plug is attached to the remote interlock ① or that is connected to your equipment and the interlock is released.
- Set the power switch ② to ON.
- Turn the key ③ to the ON position.
- Set the shutter switch ④ OPEN.

CAUTION **The red lighting of the LASER EMISSION LED of the LD405/440 laser power supply indicates that the laser is oscillating. With a certain setup, the laser beam is output by simply setting the shutter switch ④ to ON.**

4.5 LD559 laser: FV10-LD559



FV10-LD559 laser power supply

CAUTION **Be careful as a stronger laser beam that the rated power may be emitted during calibration.**

CAUTION **Temperature error would occur if LASER key switch is turned OFF during calibration. In such a case, turn POWER switch to OFF and start the system again.**

CAUTION **In an event that a power control error (PL lamp lit in orange) occurred while the system is in use, turn both LASER key switch and POWER switch to OFF position and start the system again.**

- Verify that the remote interlock ① is set properly.
- Connect the power cord with power receptacle.
- Turn POWER switch ② to **|** - (ON). TEMP lamp will blink in green and temperature adjustment will take place. When the temperature adjustment is completed, the blinking of TEMP lamp will turn to lighting. Hold on (about 3 minutes) until the adjustment is completed.
- Insert the provided key into LASER key switch ③ and turn it ON. LASER lamp will blink in red and calibration[1] will take place. When the calibration is completed, the blinking of LASER lamp will turn to lighting and a laser beam will be emitted in stable output condition. Hold on (about 2 minutes) until the calibration is completed. (When LASER key is turned ON, first time, after POWER switch is turned ON, the calibration will be executed.)

[1] About calibration

Calibration is performed to adjust the setting temperature of optical element inside the laser head to optimal condition.

1-2 Starting the Software

CAUTION **Turn on the microscope and power supply units before starting this software.**

1. Enter the user name and password to log in the Windows.

CAUTION **Log in using the user name given the Administrator's authority.**

OS	User name	Password
Windows7 (64bit)	olympus	olympus
Others	fluoview	fluoview

FV10-ASW

FV10-ASW 4.0

[FLUOVIEW] icon

2. Double-click the [FLUOVIEW] icon on the desktop.

◎ If more than one user uses the FV1200, each user should personally log in personally. For details, refer to Appendix E, "USER REGISTRATION OF FV1200" in Volume [OPERATION INSTRUCTIONS].

◎ It takes 20 to 30 seconds after the [FLUOVIEW] icon is double-clicked till the software starts up.

CAUTION

- **Images cannot be observed if the manual shutter of the fluorescence mirror unit is close. In this case, slide the shutter to the open position.**
- **When you lower the objective lens, please pay a careful attention so that the objective lens does not touch the specimen.**

1-3 Exiting from the Software

Exit from the application software and shut down Windows.

CAUTION **After exiting the application software, the light of mercury burner power supply unit may exposure to specimen. To avoid this, perform either of the followings,**

- **Close the manual shutter of the mercury burner power supply unit.**
- **Turn off the mercury burner power supply unit.**
- **Close the manual shutter of the fluorescence mirror unit (BX61WI or IX81).**

1-4 Turning the Power Off

Order to set the power switches of the units to O (OFF) is opposite to the order of turning the power switches on.

1.Set the power switch of the laser to O (OFF).

Power turning OFF when using of Multi-line Argon laser (FV5-LAMAR-2)
Turn the key to OFF position. Set the power switch to OFF.

In case of LD559 laser
Turn LASER key switch to OFF position and then, turn POWER switch to O – (OFF) position.

2.Set the power switches of the units of FV1200 to O (OFF).

3.Set the power switches of the units of microscope to O (OFF).
When using the IX83P2ZF microscope
◎ Set the main switches of the units to O (OFF) in the following order:
1) Main switch of the touch panel controller
2) Main switch of the IX3-CBH

4.Set the power switches of the controller and the monitor to O (OFF).

2 USING THE CONTROLS

◎ Only IX83 microscope combination is described.

2-1 Microscope Frame

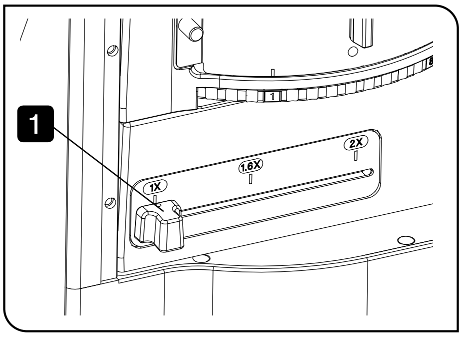
1 Motorized Revolving Nosepiece

The motorized revolving nosepiece can be rotated to switch the objective by controller U-MCZ, touch panel controller or controller.

2 Coded intermediate magnification changer IX3-CAS

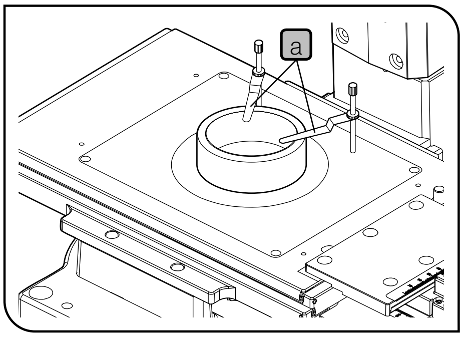
Use the coded intermediate magnification changer IX3-CAS to switch the observation magnification in the following 3 levels according to the objective lens magnification.

- 1X
- 1.6X
- 2X



1 Change the magnification by operating the changing slider.

2-2 Stage



1 Placing the Specimen

Place the specimen on the center of the stage.

◎ If the specimen is prone to slide on the stage, attach the stage clips (IX-SCL) a and clamp the specimen down with the clips.

With the mechanical stage with right handle IX3-SVR

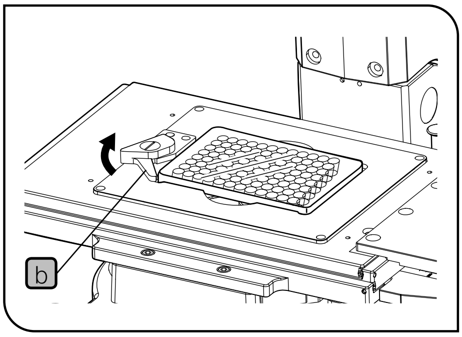
For IX3-SVR, in addition to the holder for the round stage center plate, following sample holders corresponding to each sample can be attached.

• IX3-HOW	: Microplate holder
• IX3-HOS	: Slide holder
• IX3-HO35D	: Dish holder

See page 57 for assembly.

CAUTION **The sample holder fixes the specimen to reproduce the specimen position. Do not push up the specimen by the objective lens. The specimen may be popped out.**

IX3-HOW



Open the specimen holder b of IX3-HOW, set the microplate in the center, push it toward the right diagonal direction, and return the specimen holder back to the original position.

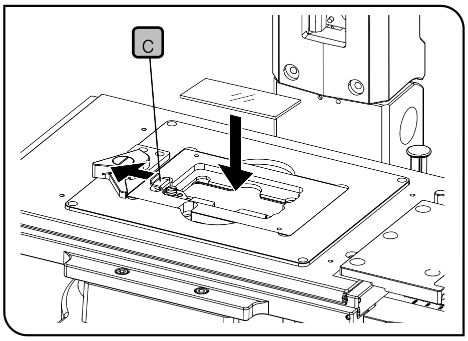
«Mountable Microplate»

Microplate compliant with SLAS (ANSI/SBS Microplate Standards issued on Jan. 9, 2004.)

Size: 127.76 (plus or minus 0.5) x 85.48 (plus or minus 0.5) mm

Specimen holder: IX3-HOS, IX3-HO35D

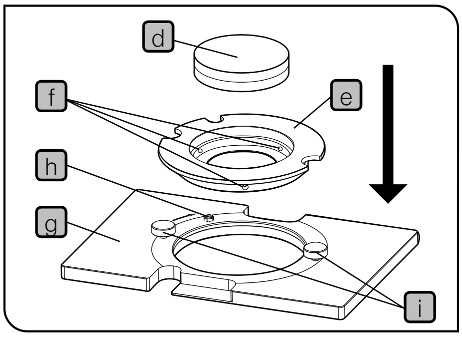
IX3-HOS



Open the specimen fixing part c of IX3-HOS outward, set the specimen in the center, push it toward the right diagonal direction, and return the specimen fixing part back to the original position.

«Chamber Slide Recommended»

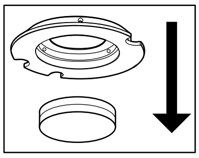
- IWAKI Chamber Slide II (76 x 26 x 0.8 to 1.0 mm)
- Nunc Lab-Tek II Chamber Slide system
  (25 x 75 x 1.2 mm)
- BD Falcon CultureSlide (25 x 75 x 1.2 mm)



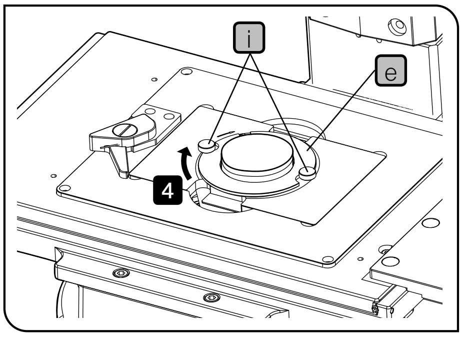
IX3-HO35D

1 Place the 35 mm dish d on the 35 mm dish fixing holder e. Tighten the fixing screws f (3 screws) placed on the side with the Allen screwdriver provided with IX3-HO35D to secure the 35 mm dish.

◎ The 35 mm dish can be secured easily by tightening the fixing screws after flipping over the 35 mm dish in advance.



CAUTION **Do not tighten the fixing screws too firmly. The dish may be damaged.**



2 Set the fixing holder e in the center of IX3-HO35D g so that the cut-out meets the holder fixing knob i.

3 Loosen the holder fixing knobs i.

4 Rotate the fixing holder e clockwise to push it to the rotation stopper h.

5 Tighten the holder fixing knobs i.

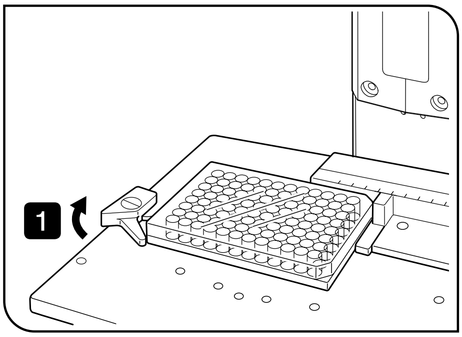
◎ The 35 mm dish fixing holder e can be sterilized by using the autoclave.

«35 mm Glass Bottom Dish Recommended»

- Matsunami Glass D111310
- MatTek P35GC-1.5-14-C

«35 mm Dish Recommended»

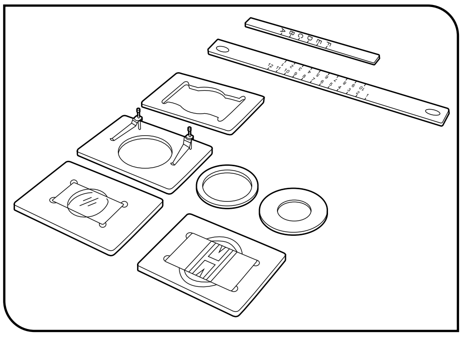
- BD Falcon 351008



With the mechanical stage IX-MVR + plain stage IX2-SP

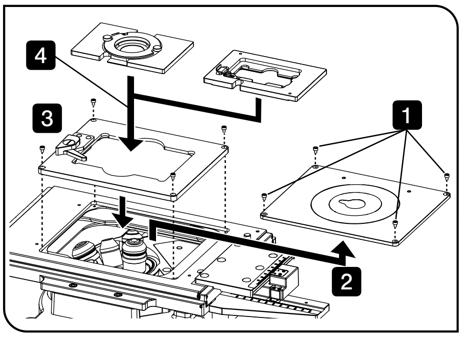
◎ 96-well or 24-well microplates, etc. are held in place by the specimen holder.
Microplates with dimensions of max. 136 mm x 92 mm can be accommodated in this way.

1 Open the spring-loaded finger of the specimen holder 1 and slide the microplate into the holder frame. Gently release the curved finger to clamp.



◎ To secure other vessels than microplates, various optional holders are available. A Terasaki plate holder is available for holding Terasaki plates (72-well, 60-well). When using this, it is necessary to replace the stage scales with those provided with the plate holder. Dish holders are available for 35 mm, 54 mm and 65 mm diameter dishes, a slide glass holder is available for holding slide glass, and the IX2-BCTP* is available for a blood cell test plate holder.
* A blood cell test plate or other calculating chamber for bacteria and eosinophil with mounting section dimensions corresponding to H 77 x V 35 x D 2 mm can be used. A 60 mm diameter dish can also be used.

Using the sample holder IX3-SVR



1 Remove the holder fixing screws (4 screws) by using the Allen wrench provided with IX3-SVR.

2 Remove the round stage center plate holder.

3 Set IX3-HOW in the center of the stage, and attach the holder fixing screws.

4 If you are using IX3-HOS or IX3-HO35D, set the sample holder in the center of IX3-HOW.

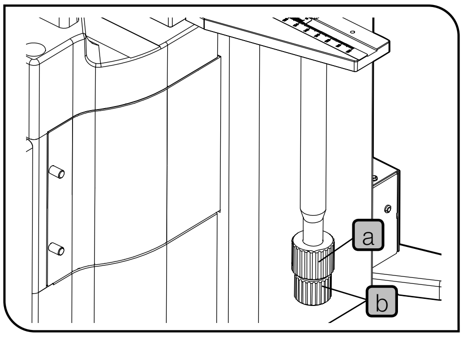
2 Moving the Specimen

CAUTION

- **Do not attempt to rotate the stage handle forcedly exceeding the stage movable range. The stage may be damaged.**
- **As the objective may interfere with the stage depending on the focus position, be sure to operate carefully.**

Stage (BX3-SSU)

The stage can be operated by the touch panel controller or the XY-Controller. As to the operation by the touch panel controller and the controller, refer to the Help of the touch panel controller and the controller (cellSens).



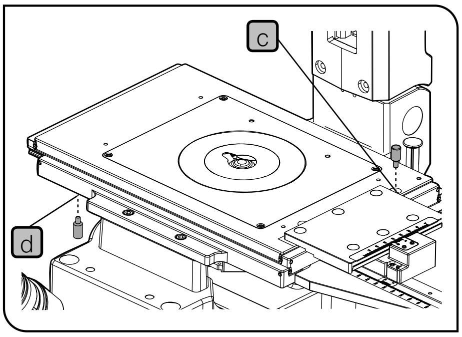
Mechanical stage with right handle (IX3-SVR)

To move the specimen to a desired position, rotate the X-axis knob a and Y-axis knob b.

◎ If the stage is used for a long time period, the stage movement range may be narrower rarely. In this case, move the stage several times within the full movement range toward the front/back or right/left direction while holding the top surface of the stage with both hands.

Movement control knob

Attaching the movement control knob provided with IX3-SVR makes it difficult to move the stage in the blocked direction. Even though you may touch the stage accidentally during observation, the observation position can be secured.



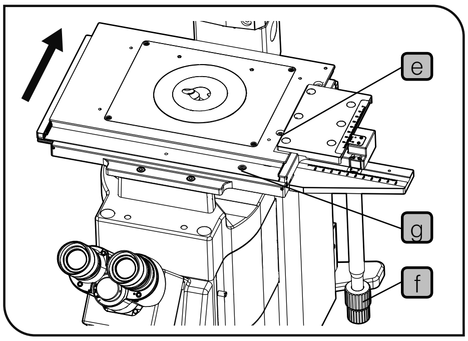
If the movement control knob is attached to the hole of c, the Y-axis movement will be blocked. If it is attached to the hole of d (backside of the stage), the X-axis movement will be blocked.

CAUTION **Do not tighten the movement control knob too firmly. The stage may be damaged.**

◎ If you tighten the movement control knob while focusing on the specimen, it will be defocused.

Movement range limit screw

As a factory default, IX3-SVR, is equipped with the movement range limit screw which limits the stage movable range in the vertical or horizontal direction.
To enlarge the movable range to observe microplates, etc., remove the movement range limit screw.



1. Remove the movement range limit screw e in the vertical direction with the Allen screwdriver provided with the microscope.
2. Rotate the longitudinal handle f to move the stage inward.
3. Remove the movement range limit screw g in the horizontal direction with the Allen screwdriver provided with the microscope.

Stage movable range

With movement range limit screw : 50 mm in vertical direction, 50 mm in horizontal direction
Without movement range limit screw: 75 mm in vertical direction, 114 mm in horizontal direction

CAUTION **When using the center plate provided with IX3-SVR, attach the movement range limit screw.**

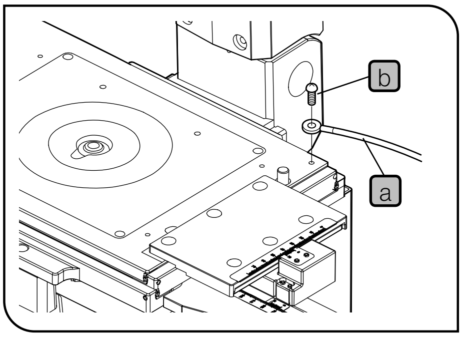
If the movement range limit screw is not attached, the objective may hit the stage.

With the Mechanical Stage IX-MVR

To move the specimen to a desired position, rotate the X-axis knob and Y-axis knob in the same manner as IX3-SVR.

◎ The stage travel area is 130 mm (X-axis) x 85 mm (Y-axis).

3 Connecting the Grounding Wire



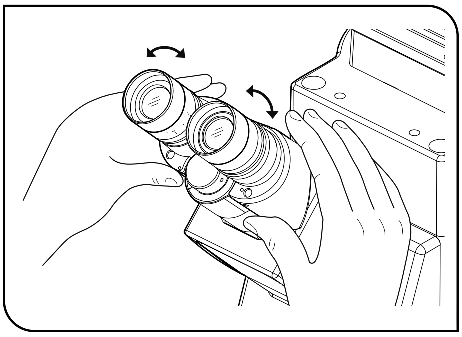
With the Stage BX3-SSU or IX3-SVR

◎ A grounding wire can be attached to the stage for electrophysiological experiments, etc.

Prepare a grounding wire a and one M4 screw b and attach the grounding wire to a screw hole on the stage surface.

CAUTION **The screw hole may sometimes be stuck by paint, etc. In such a case, screw in the M4 screw a few times to expose the metallic thread inside the screw hole and improve the contact before attaching the grounding wire firmly.**

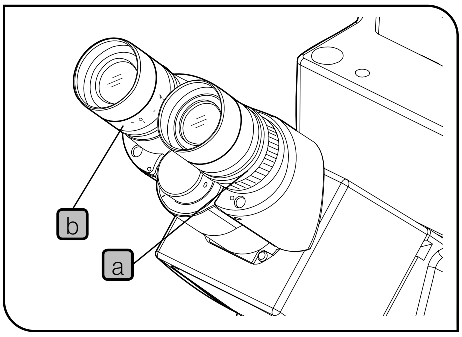
2-3 Observation Tube



1 Adjusting the Interpupillary Distance

While looking through the eyepieces, adjust the binocular vision until the left and right fields of view coincide completely. The index dot • indicates the interpupillary distance.

◎ Note your interpupillary distance so that it can be quickly duplicated



2 Adjusting the Diopter

◎ The diopter adjustment makes it possible to reduce the specimen focusing error even after the objective is switched. As the diopter varies between individuals, the diopter adjustment is required for each person.

CAUTION **The eyepiece with diopter adjustment ring should always be inserted into the observation tube without the diopter adjustment ring.**

1 Set the diopter adjustment rings on both sides to scale “0”.

2 Engage a high-power objective (e.g. 40X) in the light path, look into the right eyepiece with your right eye, and bring the sample into focus using the FOCUS button/slider of the touch panel controller.

◎ Do not use an immersion objective.

3 Engage a low-power objective (e.g. 10X) in the light path, rotate only the right diopter adjustment ring a to bring the sample into focus. At this time do not touch the FOCUS button/slider.

4 Looking into the left eyepiece with your left eye, rotate only the left diopter adjustment ring b to bring the sample into focus.

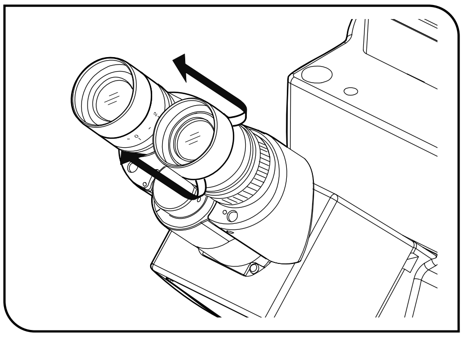
◎ The above procedure adjusts the diopter with reference to the right eye, but it is also possible to adjust with reference to the left eye. In this case, read the above procedure by inverting “right” and “left.”

Using an eyepiece including a micrometer disk

1 Looking through the eyepiece with micrometer disk, turn the diopter adjustment ring b so that the micrometer in the field of view is sharply visible.

2 Looking through the eyepiece with micrometer disk, focus on the sample using the FOCUS buttons/slider of the touch panel controller so that both the micrometer and sample are sharply visible.

3 Looking through the other eyepiece, turn only the diopter adjustment ring a to focus on the sample.



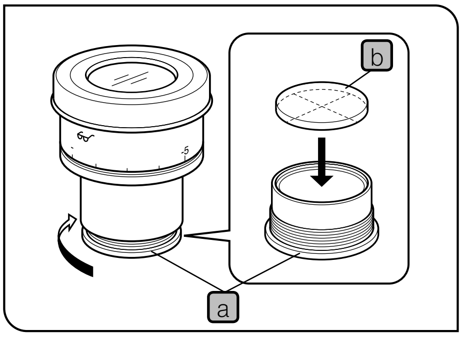
3 Using the Eye Shades

When wearing eyeglasses

Use the eye shades in the normal, folded-down position. This will prevent the eyeglasses from being scratched.

When not wearing eyeglasses

Extend the folded eye shades in the direction of the arrow to prevent extraneous light from entering between the eyepieces and eyes.



4 Mounting the Eyepiece Micrometer Disk

When the WHN10X-H eyepieces are used, an eyepiece micrometer disk can be mounted.
Use 24 mm dia. x 1.5 mm thick micrometer disks.
Turn the built-in micrometer-mounting frame a to the arrow direction (see figure) to remove it from the eyepiece and place a micrometer disk b into the mounting frame so that the surface with the model indication faces downward.

◎ The micrometer-mounting frame may be to tight for certain micrometer disks.
In this case, turn the frame by holding the circumference with a light, uniform force or by applying the frame against a rubber sheet. Do not grasp the frame with a strong force, as this may deform the frame and make it harder to remove it.
Re-attach the micrometer mounting frame in the original position.

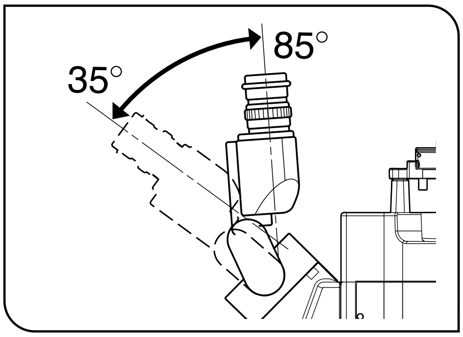
◎ Be careful not to touch the lens or micrometer surface with your finger.

5 Selecting the Light Path of the Trinocular Tube

Slide the light path selector knob to select the desired light path.

(ex.) U-TR30-2, U-TR30H-2

Light path selector knob position		
Pushed in	Middle position	Pulled out
Observation 100%	Observation 20% Camera 80%	Camera 100%



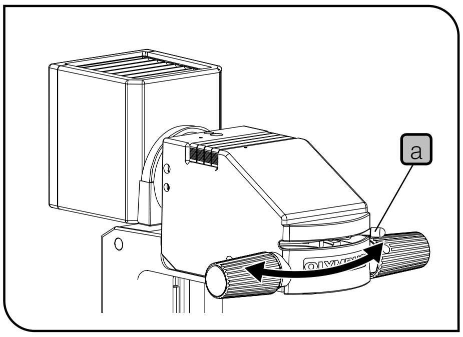
6 Adjusting the Tilt (U-TBI90)

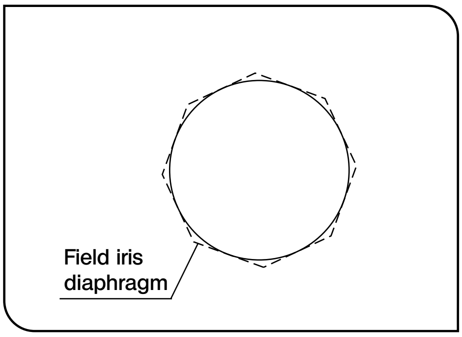
◎ Adjust the height and tilt of the eyepieces to obtain the most comfortable viewing position.
Holding the binocular section with both hands, adjust it to the desired position.

CAUTION **Never attempt to force the binocular section past the upper or lower stop position. Applying excessive force could destroy the limiting mechanism.**

◎ If the U-TBI90 is used together with any type of Intermediate tubes attachment, the image may be cut off or obscured.

2-4 Illumination Column (IX3-ILL)





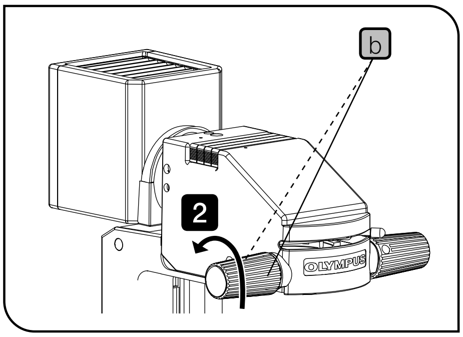
1 Using the Field Iris Diaphragm

◎ The field iris diaphragm lever is used to adjust the diameter of the illumination beam in accordance with the objective in use. Adjust the diaphragm so that the field of view is circumscribed by the field iris diaphragm to cut extra light and improve the contrast of images.

1 Move the field iris diaphragm lever a to the left or right to close or open the diaphragm.

○ : Direction for opening the diaphragm

⊛ : Direction for closing the diaphragm



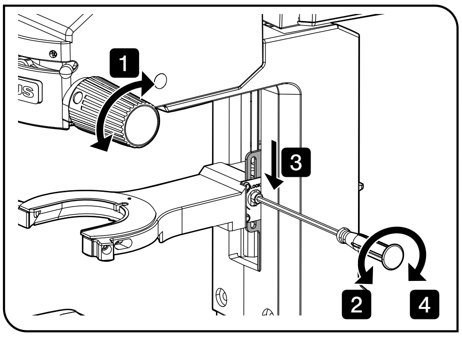
2 Adjusting the Condenser Height Adjustment Knob Tension

1 Loosen the two knob clamping screws b on the left adjustment knob using the Allen screwdriver.

2 While holding the right adjustment knob not to rotate it, turning the left adjustment knob counterclockwise (in the direction of the arrow) decreases the rotation tension and clockwise increases it. Rotating the right adjustment knob allows adjusting the tension of the condenser height adjustment knob while checking it.

3 After adjustment, tighten the two knob clamping screws b securely.

3 Condenser refocusing stopper



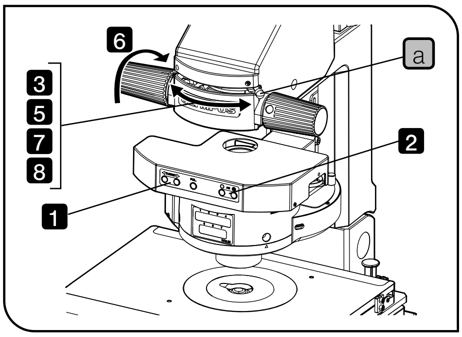
A mechanism returns the condenser back to the original position easily after moving the condenser.

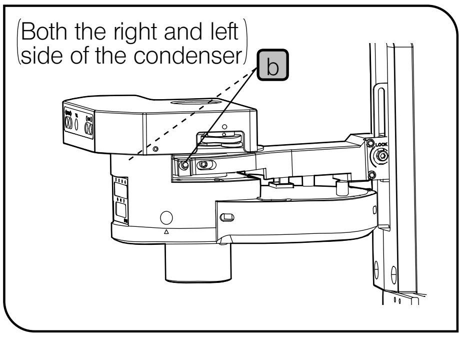
1. Bring the field diaphragm image into focus by rotating the condenser height adjustment knob.
2. Loosen the clamping screws of the stopper using the Allen screwdriver provided with the microscope.
3. Push the top of the stopper downward so that the stopper contacts the column securely.
4. Tighten the clamping screws of the stopper using the Allen screwdriver provided with the microscope.

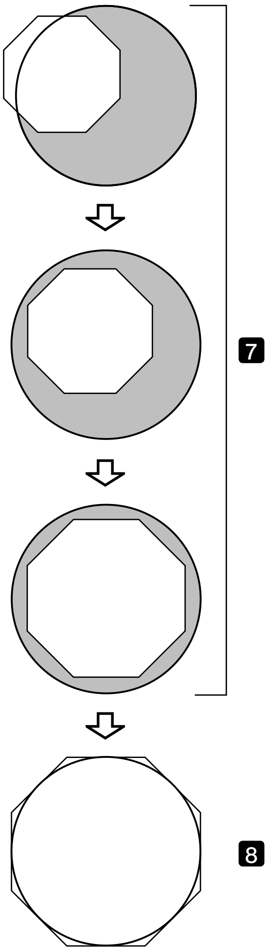
CAUTION **Rotating the condenser height adjustment knob beyond the condenser height adjustment area with an excessive force could damage the microscope. Pay careful attention when rotating it.**

◎ If the manipulator is assembled to the column, the Allen screwdriver provided with the microscope may not be used in some cases.

2-5 Condenser







1 Centering the Condenser

With the Condenser (IX3-LWUCDA, IX2-LWUCD, IX-ULWCD or IX2-MLWCD)

1 Rotate the turret (either manually or electrically) to select the "BF" brightfield observation (with which no optical element is engaged in the light path).

2 Move the aperture iris diaphragm lever to open the diaphragm.

3 Move the field iris diaphragm lever a to the fully open position (⊛ → ○).

4 Engage the 10X objective and bring the specimen into focus.

5 Using the field iris diaphragm lever a, stop down the field iris diaphragm until its image is just inside the field of view.

6 Rotate the condenser height adjustment knob to bring the field iris diaphragm image into focus.

7 While gradually opening the field iris diaphragm lever a, install the Allen screwdriver provided with the microscope in the adjustment hole b and rotate it so that the field iris diaphragm image is centered in the field of view of the eyepieces.

8 To check centration, open the field iris diaphragm lever a until its image inscribes the field of view. Now the condenser is centered.

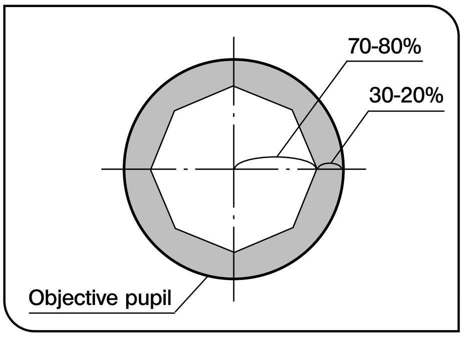
In actual observation, open the field iris diaphragm until its image circumscribes the field of view.

Effect of Field Iris Diaphragm

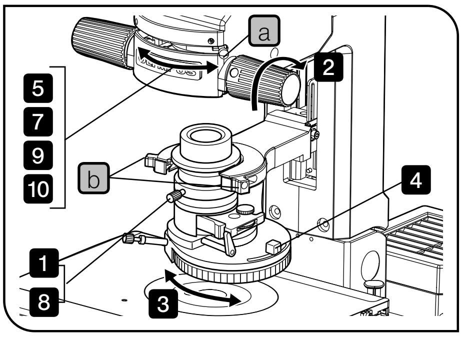
This is the iris diaphragm to adjust the area to be illuminated. By narrowing down to the level circumscribing the field of view depending on objectives, the excess light can be shielded to acquire the image with good contrast.

◎ Depending on the condenser to be used, the field iris diaphragm may not be viewed by the objective of 40X or higher magnification.

2 Using the Aperture Iris Diaphragm



- ◎ In general, the potential resolving power of an objective is fully utilized if the diaphragm is stopped down to correspond with the numerical aperture (N.A.) of the objective.
- ◎ Depending on the specimen, image contrast or focal depth in observation or acquisition may be improved by keeping the aperture iris diaphragm stopped down a little. In general, a good image is obtained if the diaphragm is stopped down to between 70% and 80% of the N.A. of the objective. Stop further down for less contrasty specimens.
- ◎ To check the position of the perimeter of the aperture iris diaphragm, remove the eyepieces and look into the eyepiece sleeves to view the aperture iris diaphragm image and the objective's exit pupil.



With the U-UCD8 Condenser

1. Rotate the condenser height fine adjustment knob counterclockwise to loosen it, then push the knob all the way toward the rear.
2. Rotate the condenser height adjustment knob in the direction of the arrow to lower the condenser to the level not to hit the specimen.
3. Rotate the turret to select the “BF” brightfield observation (with which no optical element is engaged in the light path).
4. Move the aperture iris diaphragm lever to open the diaphragm.
5. Move the field iris diaphragm lever a to the fully open position (⊛ → ○).
6. Engage the 10X objective and bring the specimen into focus.
7. While gradually opening the field iris diaphragm lever a, install the Allen screwdriver provided with the microscope in the adjustment hole and rotate it so that the field iris diaphragm image is centered in the field of view of the eyepieces.
8. Slowly move the condenser height fine adjustment knob toward the front to bring the field iris diaphragm image into focus. When focusing is obtained, rotate the knob clockwise to clamp it.
9. While gradually opening the field iris diaphragm lever a, install the Allen screwdriver provided with the microscope in the adjustment hole b and rotate it so that the field iris diaphragm image is centered in the field of view of the eyepieces.
10. To check centration, open the field iris diaphragm lever a until its image inscribes the field of view. Now the condenser is centered.

◎ In actual observation, open the field iris diaphragm until its image circumscribes the field of view.

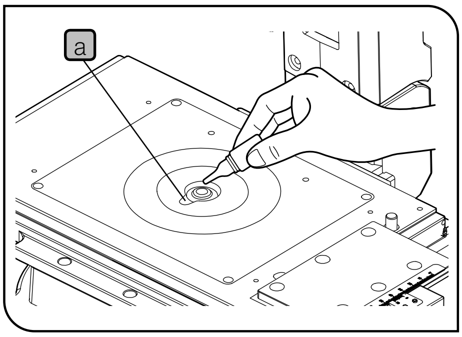
◎ When replacing the specimen or spreading immersion oil, use the condenser height adjustment knob to raise the condenser first. After finishing the procedure, lower the condenser to its lowest position.

2-6 Oil- or Water-Immersion Objective

1 Using Oil- or Water-Immersion Objective

◎ If you use an oil-immersion objective, use immersion oil as described below.

CAUTION **Always use immersion oil supplied by Olympus.**



1 Using a low-power objective, bring the specimen into focus.

2 Rotate the revolving nosepiece to engage the oil immersion objective.

3 Remove the specimen and move the stage insert cut-out a close to the objective front lens. Apply a drop of the immersion oil to the objective front lens. Place the specimen and rotate the fine adjustment knob to bring the specimen into focus.

CAUTION
- **Use as little oil as possible.**
- **If the oil contains air bubbles, the image will be degraded. Make sure the oil is free of air bubbles.**

4 After use, remove immersion oil from the objective front lens by wiping with gauze slightly moistened with absolute alcohol.

◎ The presence of air bubbles can be checked by viewing the pupil of the objective (viewed as a bright circular shape) in the tube after removing the eyepiece and opening the field iris diaphragm and the aperture iris diaphragm completely.

CAUTION **Caution in use of immersion oil:**

If immersion oil enters your eyes or contacts your skin, immediately take the following treatment.

Eyes: Rinse with fresh water (for 15 minutes or more).
Skin : Rinse with water and soap.

If the appearance of the eyes or skin is altered or pain persists, immediately see your doctor.

3 OTHER OBSERVATION METHODS

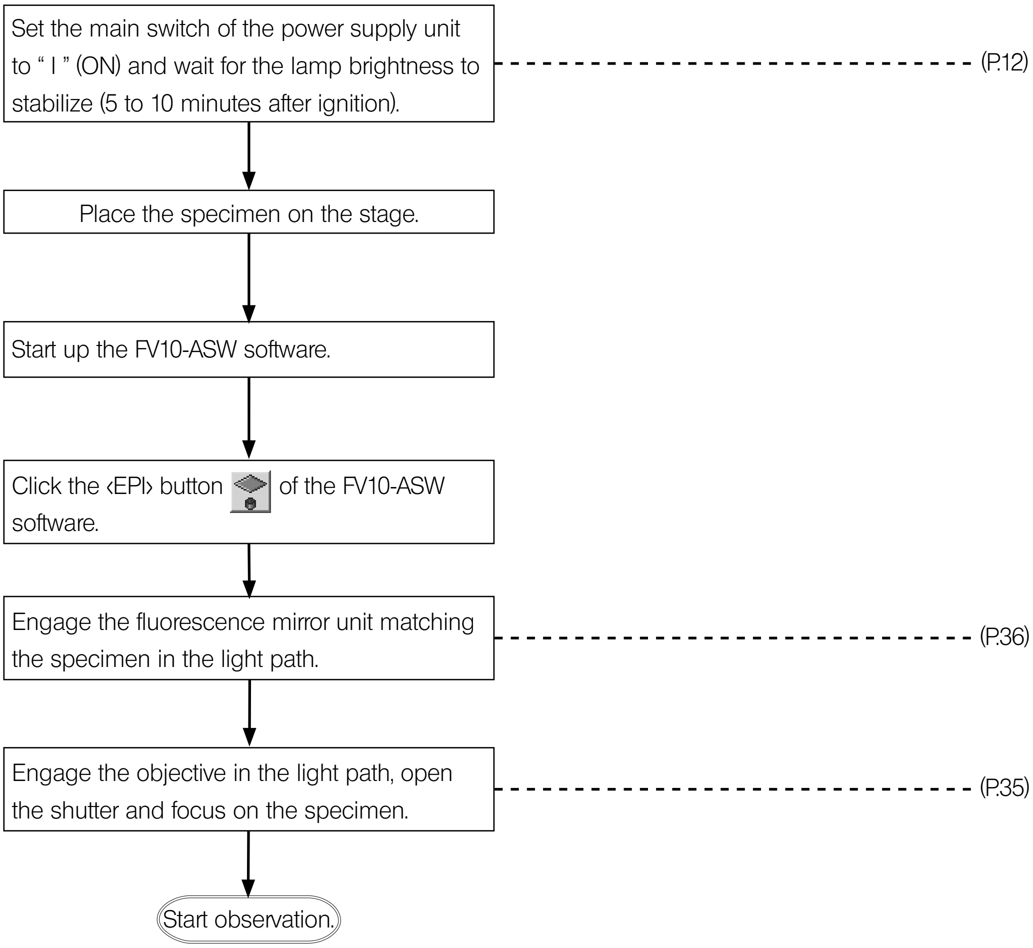
3-1 Reflected Fluorescence Observation

1 REFLECTED FLUORESCENCE OBSERVATION PROCEDURE

◎ If you need simultaneous observations of reflected fluorescence observation with the phase contrast observation or transmitted light Differential Interference Contrast (DIC) observation, please read Chapter 5, “SIMULTANEOUS FLUORESCENCE OBSERVATION” (page 44).

Preparation (Page)

- Attach the fluorescence mirror unit and objective matching the observation method.
- Set the main switch of related control boxes to “ I ” (ON).



Set the main switch of the power supply unit to “ I ” (ON) and wait for the lamp brightness to stabilize (5 to 10 minutes after ignition). (P.12)

Place the specimen on the stage.

Start up the FV10-ASW software.

Click the ‹EPI› button of the FV10-ASW software.

Engage the fluorescence mirror unit matching the specimen in the light path. (P.36)

Engage the objective in the light path, open the shutter and focus on the specimen. (P.35)

Start observation.

◎ Engage the shutter if you take a short break during the observation. (P.35)

2 General Precautions for Observation

1. Make sure that the power cord and connecting cables are plugged in securely.
2. If you perform only transmitted phase contrast or transmitted DIC observations*, leave one position on the turret empty.
   This allows for transmission of white light and reproduction of original colors.
   * This care is not required when the transmitted DIC mirror unit IX3-FDICT with built-in analyzer is used.
3. Always use Olympus immersion oil for oil immersion objectives.
4. If you use an objective with correction collar, you can correct contrast degradation due to variation in cover glass thickness by adjusting the correction collar.

Correction procedure

If the cover glass thickness is known, match the correction collar to the cover glass thickness using the collar scale provided. If the thickness is not known, turn the collection collar and adjust the fine adjustment knob to where the image contrast is best.

5. Engage the shutter if you take a short break during the observation.
6. Photobleaching of specimens
   This system features high excitation light intensity to ensure bright observation of dark fluorescence specimens.
   In consequence, after long period of observations using high-power objectives, the fluorescence of specimens will bleach quicker than usual, causing the view (contrast) of fluorescent images to deteriorate.
   In such a case, slightly reduce the excitation light intensity to slow photobleaching down and to improve the fluorescence images.
   To reduce the excitation light intensity, use ND filters or aperture iris diaphragm as far as the observation is not affected or use the shutter to limit the exposure of specimen to more than necessary light.
   Commercially-marketed photobleaching protection agent (DABCO, etc.) can also delay photobleaching of specimen. The use of photobleaching protection agent is recommended especially when you perform high-magnification observations frequently.
   ◎ Remember that the photobleaching protection agents cannot be used with certain kinds of specimens.

CAUTION **When the excitation filter is removed from the fluorescence mirror unit and attached on the excitation filter slider on the side of the white lamp, very glaring light may enter the eyepieces.**

CAUTION **The mirror units provided at the No. 1 and No. 2 positions in the mirror unit cassette are used for the LSM observation. Do not use them for the other purposes than the LSM observation. Also, do not remove them from the cassette.**

3 Selecting the Fluorescence Mirror Unit

Select the fluorescence mirror unit which matches the fluorochrome in use.

◎ Usage according to the excitation light bandwidth:
Depending on the bandwidth of excitation wavelength, several combinations of the excitation filters and barrier filters are available. The wide-band (W) set is normally used. However, when the fluorescence emitted from substances other than the fluorescent stain is strong, the narrow-band (N) set is recommendable (though the fluorescence becomes slightly darker).

Dichroic mirror and filter configurations of fluorescence mirror units

Excitation method	Mirror unit	Excitation filter	Barrier filter	Dichroic mirror	Applications
U	U-FUW	BP340-390	BA420IF	DM410	• Autofluorescence observation • DAPI: DNA staining • Hoechst 33258, 33342: Chromosome
	IX3-FUWXL				
	U-FUN	BP360-370			
V	U-FVN	BP400-410	BA460IF	DM455	• Catecholamine observation • Serotonin observation • Tetracyline: Bones, teeth
BV	U-FBVW	BP400-440	BA460IF	DM455	• Quinacrine, quinacrine mustard: Chromosome • Thioflavine S: Lymphocyte • Acriflavine: Nucleic acid • ECFP
	IX3-FBVWXL				
B	U-FBW	BP460-495	BA510IF	DM505	• FITC: Fluorescent antibody • Acridine orange: DNA, RNA • Auramine: Tubercle bacillus • EGFP, S65T, RSGFP
	U-FBN	BP470-495			
G	U-FGW	BP530-550	BA575IF	DM570	• Rhodamine, TRITC: Fluorescent antibody • Propidium iodide: DNA • RFP
	IX3-FGWXL				
Y	U-FYW	BP540-585	BA600IF	DM595	Texas Red: Fluorescent antibody

Combinations for color separation

U	U-FUNA	BP360-370	BA420-460	DM410	For observing only the U-excitation stain when using U-excitation stain together with FITC.
B	U-FBWA	BP460-495	BA510-550	DM505	For observing only the B-excitation stain when using B-excitation stain with TRITC or Texas Red.
	U-FBNA	BP470-495			
G	U-FGWA	BP530-550	BA575-625	DM570	For observing only the G-excitation stain when using G-excitation stain together with Cy5.
	U-FGNA	BP540-550			

Exclusive combinations for fluorescent proteins

CFP	U-FCFP	BP425-445CFP	BA460-510CFP	DM455CFP	For ECFP.
	IX3-FCFPXL				
GFP	U-FGFP	BP460-480GFP	BA495-540GFP	DM490GFP	For EGFP
	IX3-FGFPXL				
YFP	U-FYFP	BP490-500YFP	BP515-560YFP	DM515YFP	For EYFP
	IX3-FYFPXL				
RFP	U-FRFP	BP535-555	BA570-625	DM565	For RFP
	IX3-FRFPXL				
mCherry	U-FMCHE	BP565-585	BA600-690	DM595	For mCherry
	IX3-FMCHEXL				

4 Objectives for Various Observation Methods

Objective		Reflected fluorescence						Phase contrast (Phase contrast ring)	DIC		
									U-UCD8		IX3-LWUCDA IX2-LWUCD
		340	U	V	BV	B	G		U-TLO	U-TLD	
UPLSAPO	4X	△	○	○	○	○	○	—	—	—	—
	10X2	△	○	○	○	○	○	—	—	○	○
	20X	△	○	○	○	○	○	—	○	○	○
	20XO	△	○	○	○	○	○	—	○	○	○
	30XS	△	○	○	○	○	○	—	—	○	○
	40X2	△	○	○	○	○	○	—	○	○	○
	60XO	△	○	○	○	○	○	—	○	○	○
	60XW	△	○	○	○	○	○	—	○	○	○
	60XS	△	○	○	○	○	○	—	○	○	○
	100XO	△	○	○	○	○	○	—	○	○	○
UPLFLN	4X	△	○	○	○	○	○	○*(PhL)	—	—	—
	10X2	△	○	○	○	○	○	○*(Ph1)	—	○	○
	20X	△	○	○	○	○	○	○*(Ph1)	—	○	○
	40X	△	○	○	○	○	○	○*(Ph2)	○	○	○
	40XO	△	○	○	○	○	○	—	○	○	○
	60X	△	○	○	○	○	○	—	○	○	○
	60XOI	△	○	○	○	○	○	○*(Ph3)	○	○	○
	100XO2	△	○	○	○	○	○	○*(Ph3)	○	○	○
	100XOI2	△	○	○	○	○	○	—	○	○	○
PLAPON	60XO	—	△	○	○	○	○	—	○	○	○
	60OSC	—	△	○	○	○	○	—	○	○	○
CPLFLN	10XPH	△	△	△	△	△	△	○*(PhC)	—	—	○
LCACHN	20X	—	△	△	△	○	○	○*(PhC)	—	—	—
	40X	—	—	—	—	○	○	○*(Ph2)	—	—	—
LUCPLFLN	20X	○	○	○	○	○	○	○*(Ph1)	○	○	○
	40X	○	○	○	○	○	○	○*(Ph2)	○	○	○
	60X	△	○	○	○	○	○	○*(Ph2)	○	○	○
UAPON	20XW340	○	○	○	○	○	○	—	○	○	○
	40XW340	○	○	○	○	○	○	—	○	○	○
	40XO340	○	○	○	○	○	○	—	○	○	○

○ : Recommended combination.
○* : A phase contrast objective (Ph) is required for phase contrast observation.
△ : Usable but image may be dark.
− : Not available.

5 Turning the Lamp On

Set the main switch to " I " (ON). The illumination light will stop flickering and stabilize in 5 to 10 minutes after ignition.

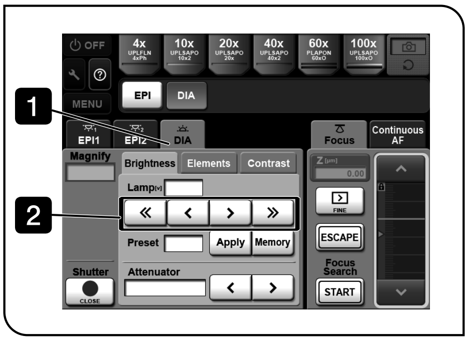
CAUTION

- **To extend the mercury burner life, do not turn it on and off at short interval. If you want to take a short break during the observation for a while in less than 2 hours after ignition, do not switch the power supply unit to off but simply close the shutter of the illuminator during a break.**
- **The mercury burner cannot be reignited until the mercury vapor has cooled down and liquefied. Before reigniting a mercury burner, wait for about 10 minutes after the last time it was turned off.**

◎ For details, see the instruction manual provided with the power supply unit.

◎ The discharge type mercury burner may not be ignited from the beginning on rare occasions due to its characteristics. In this case, set the main switch to " O " (OFF), wait for 5 to 10 seconds, then set it again to " I " (ON).

◎ After replacing the mercury burner, reset the hour counter by holding its reset button till "0.0" is displayed.


1
2
OFF
4x UPLFLN 4xPh
10x UPLSAPO 10x2
20x UPLSAPO 20x
40x UPLSAPO 40x2
60x PLAPON 60xO
100x UPLSAPO 100xO
MENU
EPI
DIA
EPI1
EPI2
DIA
Focus
Continuous AF
Magnify
Brightness
Elements
Contrast
Lamp[V]
«
‹
›
»
Preset
Apply
Memory
Shutter
CLOSE
Attenuator
Z [µm]
0.00
FINE
ESCAPE
Focus Search
START

Brightness control by using touch panel controller

1. Tap the DIA .

   DIA: Transmitted light observation

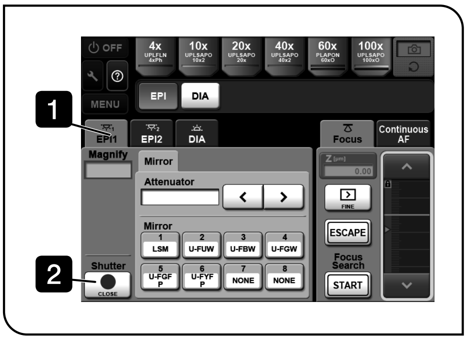
2. Tap the brightness adjustment buttons.

6 Opening/closing the shutter

When using the IX3-RFACA with IX83

The shutter can be opened and closed by the FL SHUTTER button of the touch panel controller and the U-MCZ. The open/close switching condition of the shutter can be checked by the touch panel controller

◎ For details, see the Help provided with touch panel controller.


1
2
OFF
4x
10x
20x
40x
60x
100x
MENU
EPI
DIA
EPI1
EPI2
DIA
Focus
Continuous AF
Magnify
Mirror
Attenuator
Mirror
1 LSM
2 U-FUW
3 U-FBW
4 U-FGW
5 U-FGFP
6 U-FYFP
7 NONE
8 NONE
Shutter
CLOSE
Z [µm]
0.00
FINE
ESCAPE
Focus Search
START

When using the touch panel controller

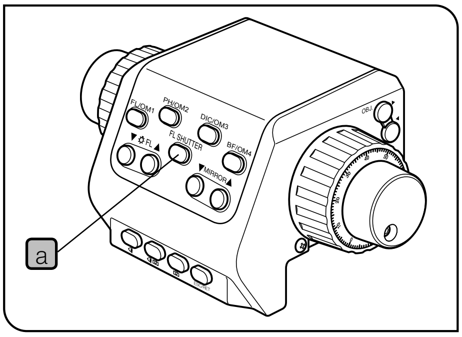
1. Tap the EPI1 .

   FL1: Observation by using 1st deck

2. Tap the Shutter .

   Blue: The shutter is closed.

   White: The shutter is opened.


a
FL/DM1
PH/DM2
DIC/DM3
BF/DM4
FL SHUTTER
FL
MIRROR
OBJ

When the U-MCZ is in use

Press the FL SHUTTER button a of the U-MCZ.

◎ The FL SHUTTER button a opens/closes the reflected shutter only. The transmitted shutter opens/closes automatically depending on the observation modes.

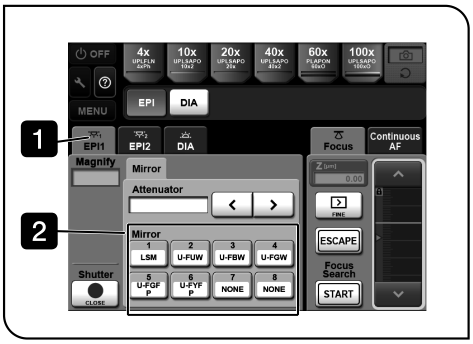
7 Switching over the mirror units in the mirror turret

When using the IX3-RFACA with IX83

The mirror units can be switched over and engaged in the light path by the touch panel controller and the U-MCZ.

◎ The display panel shows the turret number engaged in the light path of either 1st deck. You can select which turret number of the deck is displayed by the touch panel controller. The factory default is set to 1st deck.

◎ For details, see the instruction manual provided with the IX3-CBH, or Help provided with touch panel controller.

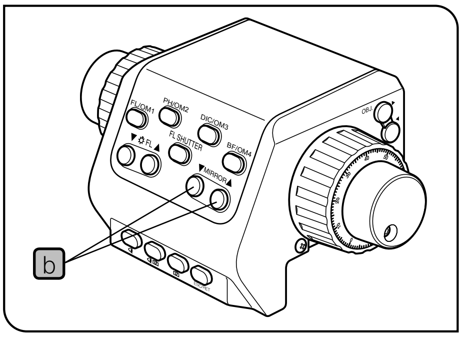

OFF
4x UPLFLN 4xPh
10x UPLSAPO 10x2
20x UPLSAPO 20x
40x UPLSAPO 40x2
60x PLAPON 60xO
100x UPLSAPO 100xO
MENU
EPI
DIA
1
EPI1
EPI2
DIA
Focus
Continuous AF
Magnify
Mirror
Attenuator
Z [μm]
0.00
FINE
2
Mirror
1 LSM
2 U-FUW
3 U-FBW
4 U-FGW
5 U-FGFP
6 U-FYFP
7 NONE
8 NONE
ESCAPE
Focus Search
START
Shutter
CLOSE

When using the touch panel controller

1. Tap the EPI1.

   FL1: Observation by using 1st deck

2. Tap the number of turret to be used.


FL/OM1
PH/OM2
DIC/OM3
BF/OM4
FL
FL SHUTTER
MIRROR
OBJ
b

When using the U-MCZ

Press the MIRROR button b of the U-MCZ.

◎ U-MCZ can switch over the turret of either 1st deck.

3-2 Phase Contrast Observation

- ◎ A phase contrast objective, phase contrast optical element, and the U-CT30-2 centering telescope are required for phase contrast observation.
- ◎ If a DIC slider, analyzer or polarizer is engaged in the light path, disengage it.

1 Phase Contrast Optical Elements and Applicable Objectives

With the IX3-LWUCDA or IX2-LWUCD

◎ Insert the optical element (small) in the 30 mm position and the optical element (large) in the 38 mm position. When observing the specimens in wells, it is recommended to use the IX-PHC to obtain the phase contrast effect in a wide range of field of view.

Optical Element	Indication	Applicable Objectives
IX-PHL (small)	PhL	UPLFLN4XPH
IX-PHC (small)	PhC	CPLN10XPH, LCACHN20XPH, CPLFLN10XPH
IX-PH1 (small)	Ph1	UPLFLN10X2PH, UPLFLN20XPH, LUCPLFLN20XPH
IX-PH2 (small)	Ph2	UCPLFLN20XPH, UPLFLN40XPH, LUCPLFLN40XPH, LUCPLFLN60XPH, LCACHN40XPH
IX-PH3 (large)	Ph3	PLAPON60XOPH, UPLFLN60XOIPH, UPLSAPO100XOPH, UPLFLN100XO2PH

Example of attaching optical elements (IX3-LWUCDA)

Small Diameter: PH1, PHL, free or PH2, C, free

Large Diameter: PH3, DIC40, DIC60, DIC100

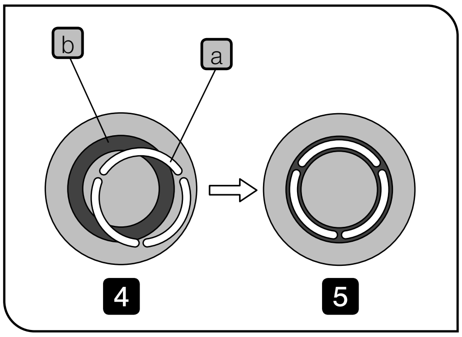
With the IX-ULWCD

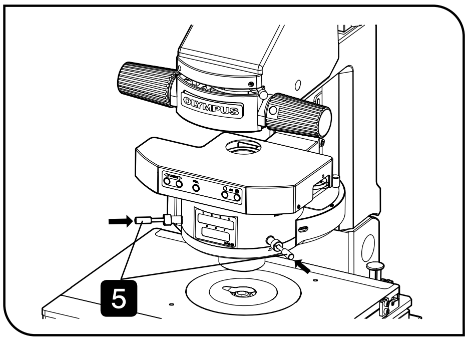
◎ The IX-PHCU or IX-PH1U can be attached only in the Ph1 and PhC. (Do not remove the built-in elements.)

Optical Element	Indication	Applicable Objectives
PHL (built-in)	PhL	UPLFLN4XPH
IX-PHCU	PhC	CPLN10XPH, LCACHN20XPH, CPLFLN10XPH
IX-PH1U	Ph1	UPLFLN10XPH, UPLFLN20XPH, LUCPLFLN20XPH,
PH2 (built-in)	Ph2	UCPLFLN20XPH, UPLFLN40XPH, LUCPLFLN40XPH, LUCPLFLN60XPH, LCACHN40XPH

◎ When using the U-UCD8 or IX2-MLWCD, refer to the provided instructions.

2 Centering the Phase Contrast Ring Slit





◎ Open the aperture iris diaphragm during phase contrast observation.

1. Engage the phase contrast objective in the light path and bring the specimen into focus.
2. Remove an eyepiece and attach the U-CT30-2 centering telescope in place.
3. Engage the ring slit of the condenser matching the phase contrast objective in the light path.
4. Rotate the knurled section of the centering telescope to focus on the ring slit a and the phase plate b of the objective.
5. Pushing the optical element centering knobs, turn the phase contrast ring slit centering screws (in positions marked) so that the ring slit image overlaps with the phase plate of the objective.

◎ Do not release the hand suddenly while the optical element centering knobs are being pushed in. The optical element centering knobs may be popped out.

6. Remove the U-CT30-2 centering telescope and attach an eyepiece in place.

◎ If the vessel is not completely flat, it may become necessary to adjust the centering again to obtain the optimum contrast.
Adjust the centering in each objective power.

7. Adjust the field iris diaphragm so that its image circumscribes the field of view and observe the phase contrast.

◎ Engaging the green filter in the light path will improve the contrast.

◎ If you are using IX83P2ZF, a thin shading may occur in the periphery of the field of view during visual observation.

3-3 Differential Interference Contrast Observation

◎ If a plastic dish is used, the normal optical performance of DIC observation cannot be manifested due to the polarization characteristic of the dish. Use a glass dish.

◎ DIC optical elements, a DIC slider, analyzer, and polarizer are required for DIC observation.

1 DIC Optical Elements, Applicable Objectives and DIC Sliders

With the IX3-LWUCDA or IX2-LWUCD

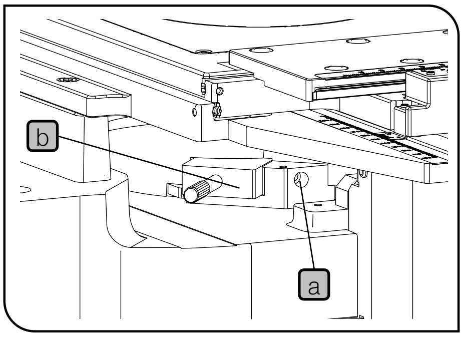
◎ Insert a small optical element (one of the optical elements inside ( ) in the following table) in the 30 mm position and other optical element (large) in the 38 mm position.

UIS2 Series

DIC Slider Applicable Objective		U-DICT	U-DICTS Shift Type	U-DICTHC High Contrast Type	U-DICTHR High Resolution Type
UPLSAPO	10X2	(IX2-DIC10)	(IX2-DIC10)	–	–
	20X	(IX2-DIC20)	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	20XO	(IX2-DIC20)	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	30XS	IX2-DIC30	IX2-DIC30	–	–
	40X2	IX2-DIC40	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	60XO	–	IX2-DIC60	–	–
	60XW	IX2-DIC60	IX2-DIC60	–	–
	60XS	IX2-DIC60	IX2-DIC60	–	–
	100XO	IX2-DIC100	IX2-DIC100	–	–
	100XOPH	IX2-DIC100	IX2-DIC100	–	–
PLAPON	60XO	–	IX2-DIC60	–	–
	60XOPH	–	IX2-DIC60	–	–
UPLFLN	10X2	(IX2-DIC10)	(IX2-DIC10)	–	–
	20X	(IX2-DIC20)	(IX2-DIC20)	(IX2-DIC20)	(IX2-DIC20HR)
	40X	IX2-DIC40	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	40XO	–	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	60X	IX2-DIC60	IX2-DIC60	–	–
	60XOI	IX2-DIC60	IX2-DIC60	–	–
	100XO2	IX2-DIC100	IX2-DIC100	–	–
	100XOI2	IX2-DIC100	IX2-DIC100	–	–
LUCPLFLN	20X	(IX2-DIC20)	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	40X	IX2-DIC40	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	60X	IX2-DIC60	IX2-DIC60	–	–
UAPON	20XW340	(IX2-DIC20)	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	40XW340	–	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	40XO340	–	IX2-DIC40	IX2-DIC40HC	IX2-DIC40HR
	100XOTIRF	IX2-DIC100	IX2-DIC100	-	-
	150XOTIRF	IX2-DIC100	IX2-DIC100	-	-
UCPLFLN	20X	(IX2-DIC20)	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
	20XPH	(IX2-DIC20)	(IX2-DIC20)	(IX2-DIC20HC)	(IX2-DIC20HR)
APON	60XOTIRF	-	IX2-DIC60	-	-
	100XHOTIRF	-	IX2-DIC100	-	-

◎ When using the U-UCD8, refer to the U-UCD8 instructions.

2 Attaching the Analyzer and DIC Slider



With the DIC Slider U-DICTS/U-DICTHC/U-DICTHR

◎ If you use these sliders, the analyzer U-ANT cannot be used. The transmitted DIC mirror unit IX3-FDICT is required.

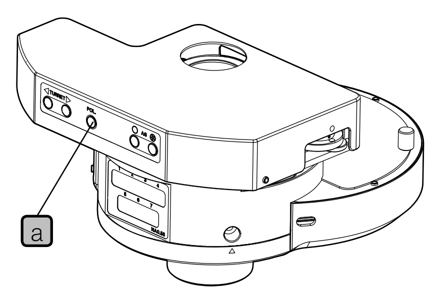
1. Loosen the fixing screw a by using the Allen screwdriver provided with the microscope to remove the dummy slider from the revolving nosepiece.
2. Insert the DIC slider b to be used into the revolving nosepiece by facing its display surface down.

With the Transmitted DIC Mirror Unit IX3-FDICT

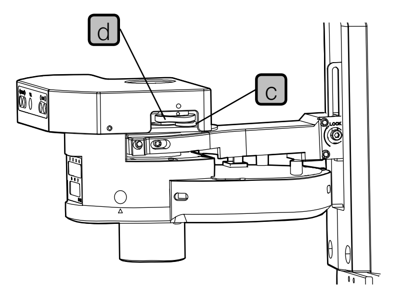
IX3-FDICT is the mirror unit equipped with the analyzer. This is attached to the mirror unit cassette and engaged into the light path during the transmitted differential interference observation.

In this case, it is not necessary to attach the analyzer U-ANT to U-DICT.

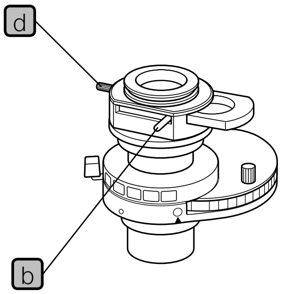
3 Cross-Nicol Adjustment



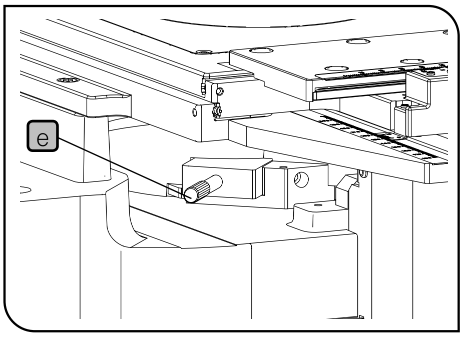
when using IX3-LWUCDA

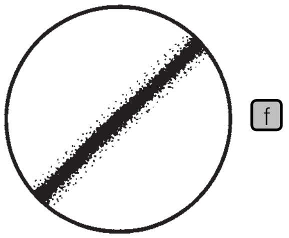


when using IX3-LWUCDA



when using IX-LWPO





1. Rotate the condenser's turret for the BF (brightfield) light path (with no optical element engaged in the light path).
2. When IX3-LWUCDA is used, push the button a of IX3-LWUCDA to engage the polarizer in the light path.
   When IX-LWPO is used, move the polarizer detaching lever b on the IX-LWPO polarizer to engage the polarizer in the light path.
3. Engage the 10X objective in the light path, place an optimum specimen for brightfield observation on the stage, bring the specimen into approximate and remove the specimen out of the light path.
4. Refer to page 40 engage the DIC slider and the analyzer in the light path.
5. Remove the eyepieces from the observation tube, look into the observation tube.
6. Move the prism movement knob e of the DIC slider in the clockwise direction around the axis until the knob is stopped. First a black interference stripe then a rainbow-colored interference stripe will be observed. Stop the knob at the position in which the black interference stripe can be seen. (State of f)
7. When IX3-LWUCDA is used, loosen the polarizer rotation knob d by rotating the clamping knob c in the clockwise direction until the knob stops.

   When IX-LWPO is used, loosen the clamping of the polarizer rotation/clamping knob by rotating slightly the polarizer rotation/clamping knob d in a counter-clockwise direction.
8. While looking into the observation tube, rotate the polarizer rotation/clamping knob d on the polarizer unit horizontally until the black interference stripe becomes darkest. This is the cross-nicol position.
9. After determining the position, clamp the polarizer.

   When the IX3-LWUCDA is used, rotate the clamping knob c in the counterclockwise direction around the axis until the knob is stopped.

   When the IX-LWPO is used, rotate the polarizer rotation/clamping knob d in a clockwise direction until the knob stops.

4 Observation Method

1. Rotate the condenser turret to engage the suitable optical element for the objective in use in the light path.
2. Engage the objective to be used in the light path.
3. Place the specimen on the stage and bring the specimen into focus by moving the objective up or down.
4. Adjust the field iris diaphragm so that its image circumscribes the field of view.
5. Adjust the aperture iris diaphragm to enhance the contrast.
6. Engage the DIC slider in the light path.
7. Move the prism movement knob of the DIC slider to select the interference color that can provide the optimum contrast in accordance with the specimen.

U-DICTS :
U-DICTHC :
U-DICTHR :
} The background interference color is continuously variable from black to light gray.

◎ With sensitive color observation using the U-UCD8, engage the UUCDTP530 1 plate (sensitive color plate) in the light path.

- Setting the background color to dark enables an observation like darkfield observation.
- Setting the background color to gray provides observation with high contrast and 3D feeling with the gray sensitive color with which the sensitivity is highest.
- Setting the background color to gray allows very small change in phase to be observed as a change in color.
- There is a directional characteristic with the detection sensitivity because of the configuration of the DIC prism. As a result, the contrast may sometimes be improved by rotating the specimen on the stage.

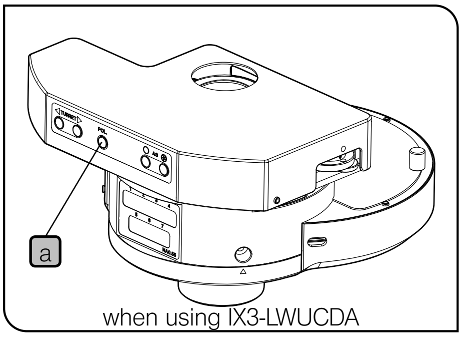
3-4 Simplified Polarized Light Observation

1 Attaching the Analyzer and Polarizer

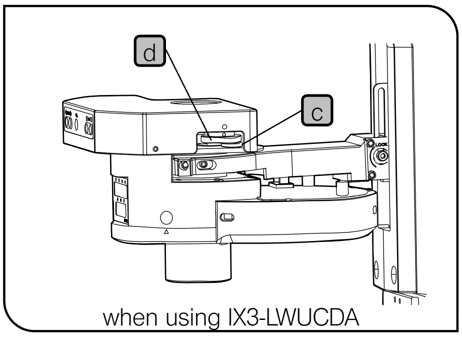
◎ Use the same procedure as that for attaching the analyzer and polarizer for DIC observation.

◎ The simplified polarized light observation is enabled also by attaching the analyzer U-ANT to the dummy slider.

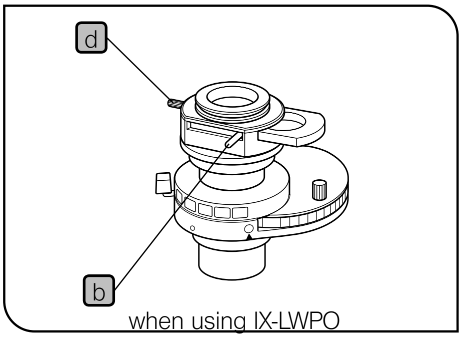
2 Observation Method



when using IX3-LWUCDA



when using IX3-LWUCDA



when using IX-LWPO

1. Rotate the turret for the BF (brightfield) light path (with no optical element engaged in the light path).
2. When IX3-LWUCDA is used, push the button a of IX3-LWUCDA to engage the polarizer in the light path.
   When IX-LWPO is used, move the polarizer detaching lever b on the IX-LWPO polarizer to engage the polarizer in the light path.
3. Rotate the revolving nosepiece to engage the objective to be used in the light path.
4. Refer to page 40 and engage the analyzer in the light path.
5. When IX3-LWUCDA is used, loosen the polarizer rotation knob d by rotating the clamping knob c in the clockwise direction until the knob stops.

   When IX-LWPO is used, loosen the clamping of the polarizer rotation/clamping knob by rotating slightly the polarizer rotation/clamping knob d in a counter-clockwise direction.
6. Move the polarizer rotation/clamping knob d on the polarizer unit horizontally in the counterclockwise direction around the axis until the position in which the field of view is darkest.
7. After determining the position, clamp the polarizer.

   When the IX3-LWUCDA is used, rotate the clamping knob c in the counterclockwise direction around the axis until the knob is stopped.

   When the IX-LWPO is used, rotate the polarizer rotation/clamping knob d in a clockwise direction until the knob stops.
8. Place a specimen on the stage and bring the specimen into focus. Now the specimen can be observed with simplified polarized light observation.
9. Adjust the field iris diaphragm so that its image circumscribes the field of view.
10. Adjust the aperture iris diaphragm to enhance the contrast.

3-5 Simultaneous fluorescence observations

◎ In case of FV10-ASW opening, Simultaneous fluorescence observations is not available.

◎ By properly combining equipment, this system can be used in transmitted light brightfield observation, transmitted light phase contrast observation and transmitted light DIC (Differential Interference Contrast) observation in addition to the reflected fluorescence observation. With specimens which bleach quickly, photobleaching can be minimized by initially using transmitted light phase contrast or transmitted light DIC observation to determine the area of the specimen to be observed. Reflected fluorescence observation can also be executed simultaneously with phase contrast or DIC observation.

1 Simultaneous reflected fluorescence/phase contrast observations

◎ The phase contrast observation requires the IX3-LWUCDA, IX2-LWUCD, IX-ULWCD, IX2-MLWCD or U-UCD8 condenser and a Ph objective.

1. Rotate the turret of the fluorescence mirror turret and engage the position without fluorescence mirror unit in the light path.
2. Rotate the phase contrast turret to show the same number as the Ph number shown on the objective.
3. Adjust the optical axis between the ring slit and phase plate by centering them.
4. Engage the mirror unit corresponding to the desired excitation in the light path and release the shutter.
5. Adjust the transmitted light for the best balance of fluorescence and phase contrast brightness.

   ◎ To adjust the transmitted light intensity, use the light intensity control knob of the microscope and ND filters in combination.
   Remember that the service life of a halogen bulb tends to decrease when the bulb is kept on continuously at a extremely low voltage, so be careful.

2 Simultaneous reflected fluorescence/DIC (transmitted) observations

◎ The DIC observation requires the following modules.

- Condenser (IX3-LWUCDA, IX2-LWUCD, IX2-MLWCD or U-UCD8)
- DIC slider (U-DICT, U-DICTS, U-DICTHC or U-DICTHR)
- Analyzer (U-ANT, IX3-AN, IX3-FDICT) *U-ANT can be mounted only on the U-DICT.

CAUTION **Do not insert the U-ANT analyzer in the DIC slider, for this will dim the fluorescence observation image and cause the analyzer to be burnt.**

1. Engage the position where the fluorescence mirror unit is not attached in the light path.
2. Adjust the polarizer on the DIC condenser to the “Crossed Nicol” (complete extinction) position.
3. Insert the DIC slider into the position provided on the revolving nosepiece.
4. Rotate the turret on the condenser to select the Nomarski prism matching the objective to be used for observation.
5. Engage the objective to be used in the light path.
6. Place the specimen on the stage and focus on the specimen.
7. Adjust the field iris diaphragm and aperture iris diaphragm.
8. Turn the prism movement knob on the DIC slider to adjust contrast of the DIC image.
9. Engage the mirror unit corresponding to the desired excitation and release the shutter.
10. Adjust the transmitted light for optimum fluorescence and DIC image brightness.

◎ If you use IX3-RFACA, each motorized module can be switched to the simultaneous observation setting status by operating the button on touch panel controller (combined with IX83).
For details, refer to Help of touch panel controller.

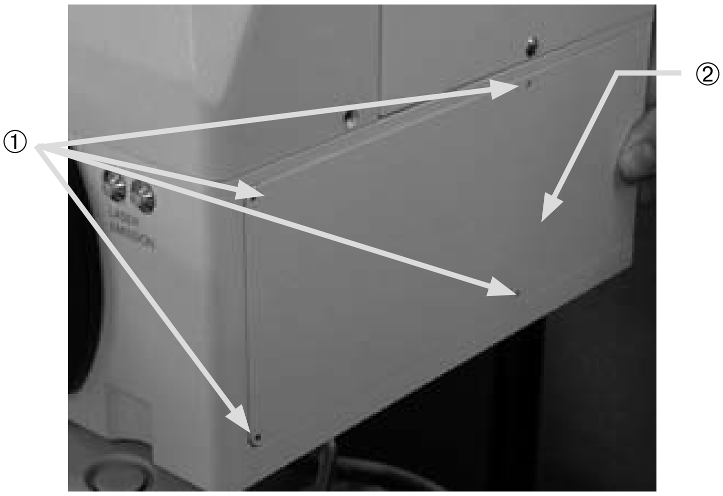
4 Replacement of Cubes

The DM (Dichroic Mirror) on the cubes is used to connect the light path of the optional FV10-ASU Auxiliary Scan Unit or FV12-HSD Non-confocal Point Detector with that of the scan unit. It should be selected according to the observation method.

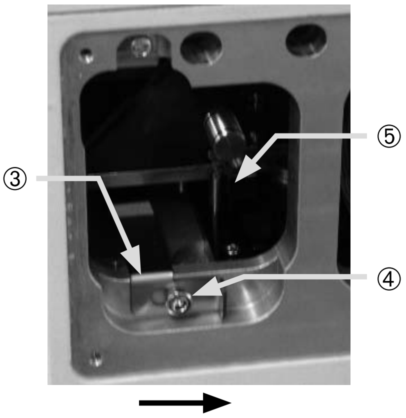
4-1 FV10-ASU

1 Taken out from FV10-ASU

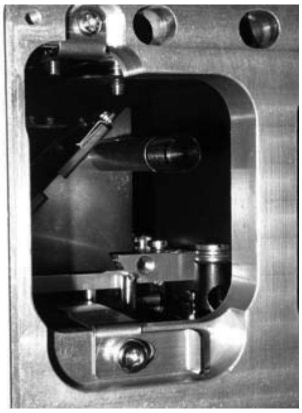
1. Set the light path of the scan unit to the LSM light path. (This can be done with the FLUOVIEW software. For details, refer to the User's Manual for the FLUOVIEW software.)
2. Loosen the four cover clamping knobs ① on the lower part of the right side panel of the scan unit, and remove the cover ②.



3. Using an Allen screwdriver, loosen the screw ④ retaining the guide lock plate ③, move the guide lock plate in the direction of the arrow, engage it with the pin ⑤ below the guide, and tighten the screw ④ again to lock the guide.

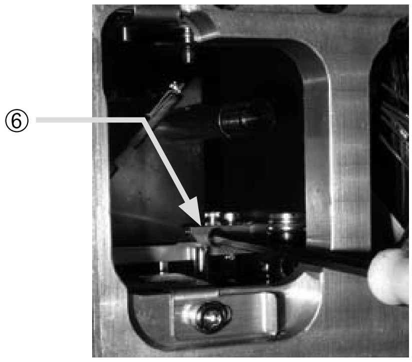


Before moving

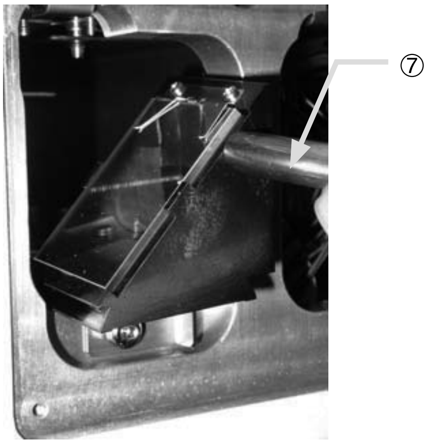


After moving

4. Using the Allen screwdriver, loosen the clamping screw ⑥ retaining the DM cube.



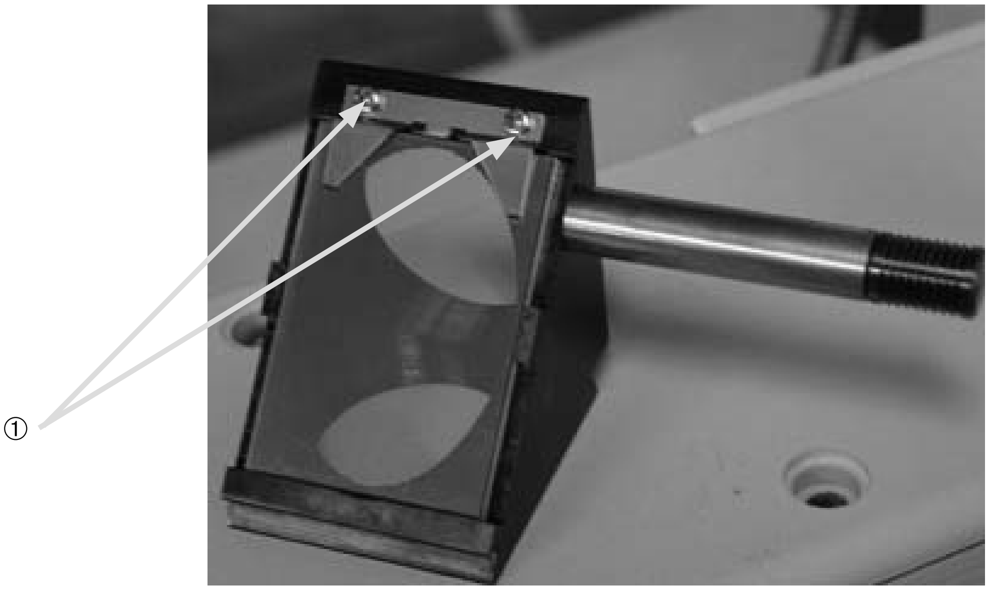
5. Pull out the DM cube insertion knob ⑦ toward you and take out the DM cube from the light path selector mechanism.



2 Fabricating the DM cube of FV10-ASU

1. Using a precision Phillips screwdriver, loosen two screws ① clamping the DM holder plate and take out the DM and DM holder plate.
2. Insert the desired DM and tighten two screws ① to clamp the DM holder plate.

◎ The applicable DM diameter is $26^{-0.1}_{-0.3} \times 38^{-0.1}_{-0.3}$ mm , with thickness of 1±0.05mm.



◎ The DM should be inserted by distinguishing the face and back. Make sure that the reflective surface (interference film surface) of the DM comes as the face.

◎ When replacing the DM cube, take care not to contaminate them with fingerprints, etc.

3 Attaching the DM cube on FV10-ASU

Attach the DM cube by reversing the removing procedure.

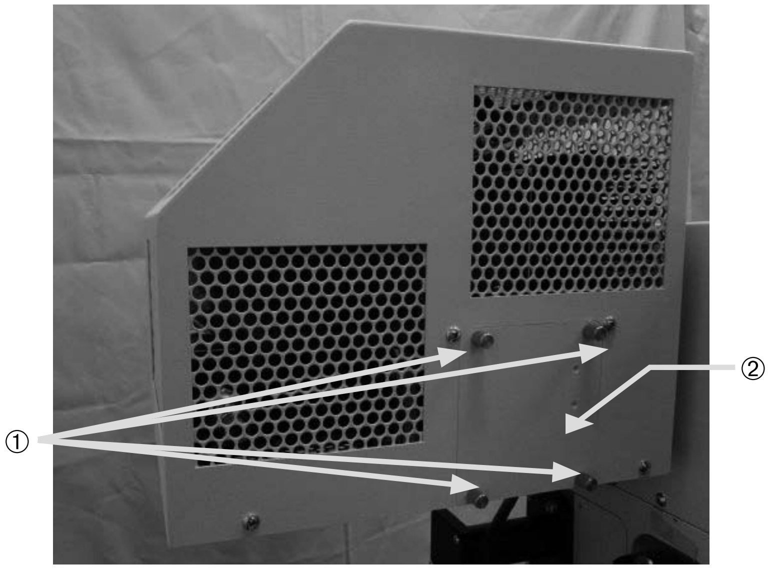
◎ To use DM cube replaced, it requires the following preparations.

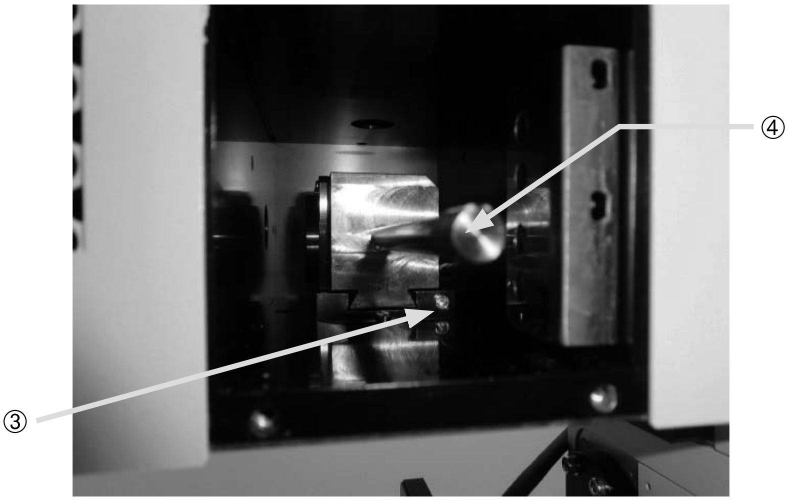
1. Log-in FV10-ASW software with the administrative right.
2. Select [Tools] – [Maintenance] and bring [FV1200 Setup] window to appear.
3. Click on ‹System Settings› button and select [Filter2] tab.
4. Verify that [Nonconfocal Detector] group box is set to “ FV10-ASU”.
5. Enter information of the spectral cube that was replaced in [Cube] drop down list, [Emission DM] text box, [BF CH1] text box and [BF CH2] text box respectively.
6. Click on ‹Save and Close› button and close [FV1200 Setup] window.
7. Re-boot FV10-ASW software.

4-2 FV12-HSD

1 Taken out from FV12-HSD

1. Using an Allen screwdriver, loosen the four cover clamping screws (provided with slip-off prevention mechanisms) ① on the side panel of the FV12-HSD and remove the cover ②.





2. Loosen the cube cover clamping screw ③ inside the cover.
3. Remove the cube cover by holding the cover knob ④.

2 Fabricating the spectral cube of FV12-HSD

A desired spectral cube can be fabricated by attaching a commercially available barrier filter and DM to the spectral cube frame.

Dimensional conditions for the optical components

Barrier filter	Ø$25^{-0.1}_{-0.3}$ mm, max. thickness 6mm or Ø$12.7^{-0.1}_{-0.3}$ mm, Thickness 2±0.05mm
Dichroic mirror	$26^{-0.1}_{-0.3}$ x $38^{-0.1}_{-0.3}$ mm, max. thickness 1.4mm or Ø$12.7^{-0.1}_{-0.3}$ mm, Thickness 2±0.05mm

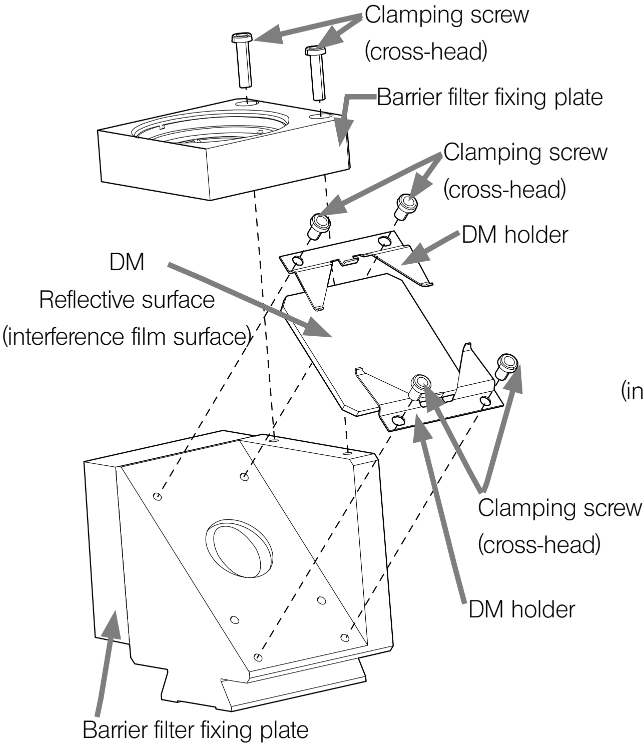
◎ When replacing the DM and barrier filter, take care not to contaminate them with fingerprints, etc.

Attaching a DM

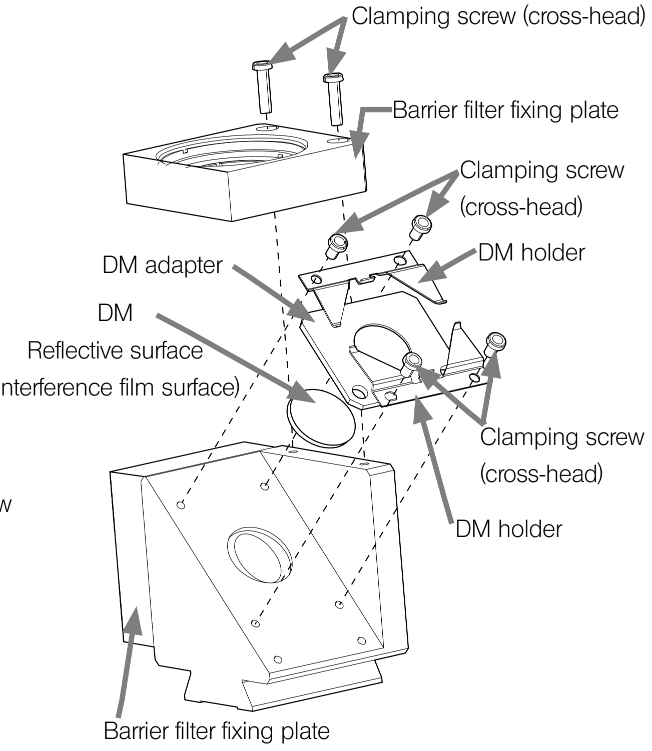
◎ Before attaching DM, detach barrier filter fixing plate on the side of DM relective surface from the spectral cube.

◎ The DM should be inserted by distinguishing the face and back. Make sure that the reflective surface (interference film surface) of the DM comes as the face.

◎ If you are using the DM Ø12.7mm, fix it with DM adapter.


Clamping screw (cross-head)
Barrier filter fixing plate
Clamping screw (cross-head)
DM holder
DM
Reflective surface (interference film surface)
Clamping screw (cross-head)
DM holder
Barrier filter fixing plate

DM 26×38 mm

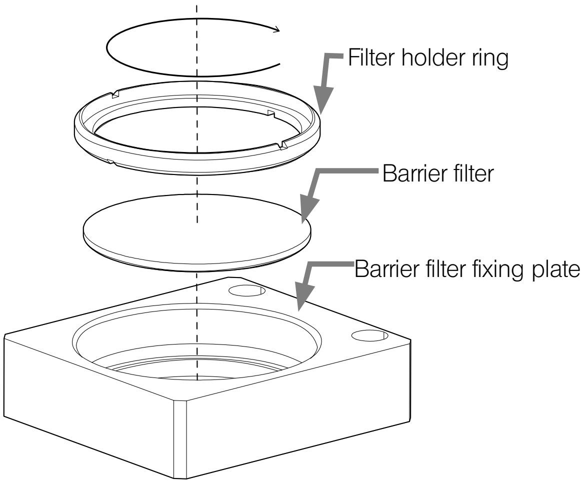

Clamping screw (cross-head)
Barrier filter fixing plate
Clamping screw (cross-head)
DM holder
DM adapter
DM
Reflective surface
(interference film surface)
Clamping screw (cross-head)
DM holder
Barrier filter fixing plate

DM Ø12.7 mm

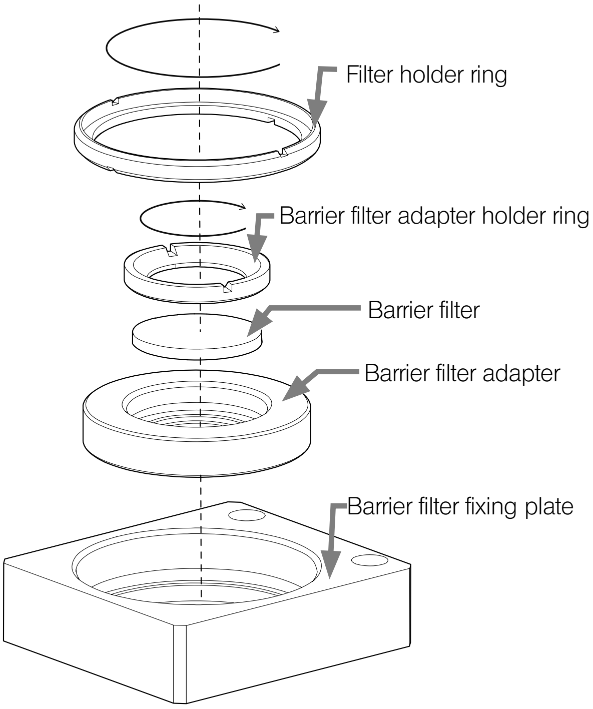
Attaching a barrier filter

◎ Insert the tip of a precision flat-blade screwdriver into the notch on the ring and turn the ring taking care not to scratch the filter to lock it.

◎ If you are using the barrier filter Ø12.7 mm, fix it with barrier filter adapter.


Filter holder ring
Barrier filter
Barrier filter fixing plate

Barrier filter Ø25 mm

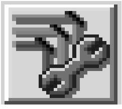

Filter holder ring
Barrier filter adapter holder ring
Barrier filter
Barrier filter adapter
Barrier filter fixing plate

Barrier filter Ø12.7 mm

3 Attaching the spectral cube on FV12-HSD

Attach the DM cube by reversing the removing procedure.

FV10-ASW



[LightPath & Dyes] button

◎ Input the information of installed DM into the FV10-ASW software.

1. Click the [LightPath & Dyes] button in the [Image Acquisition Control] window of the FV10-ASW software.
2. Input the information of installed DM using the [LightPath & Dyes] window.

5 LAMP HOUSING INSPECTION SHEET

CAUTION **The standard service life of the lamp houses are eight (8) years of use or 20,000 hours of total power ON period, whichever is the shorter period.**

- Study the instruction manual for the lamp housing before inspection.
- For safe use of the lamp housing, we recommend performing the following inspection periodically (every time you replace the lamp and at least every 6 months).
- The table below identifies the check items to be observed. Put (X) if not applicable or (✓) if applicable.
- If there is any (✓) mark noted, immediately stop use of the product, and contact Olympus for detailed inspections or replace the lamp housing.
- If you detect an abnormality other than that listed below or with other Olympus product, also stop the use of the product and contact Olympus for detailed inspections.
- Note that service, replacement and detailed inspections are charged after expiration of the warranty period.

If you have any questions, please contact Olympus.

	Check results (Date)			
Check items	/	/	/	/
1. More than 8 years have passed since original purchase or the total power ON time has exceeded 20,000 hours.				
2. Illumination flickers when you move the lamp cable or lamp housing.				
3. Lamp cable is unusually hot to the touch.				
4. Scorching or burning odor is produced during use.				
5. Deformation, backlash, or looseness, etc. when you assemble the lamp housing. (Impossibility of removing the top section of lamp housing when you attempt to replace the lamp, etc.)				
6. Discoloration, deformation or cracking of the lamp housing.				
7. Melting, crack, deformation or solidification of the lamp cable or a wiring part.				
8. Increased frequency of servicing compared to similar devices put into use at the same time as the lamp housing.				

* When the Check Result columns become insufficient, copy this sheet.

6 SPECIFICATIONS

The specifications of Microscope, IX83P2ZF, are listed in this manual.
The specifications of FV1200 systems are listed in “FV1200 User's Manual [Safety Manual]”.

Item	Specification
Optical system	UIS2 optical system
Microscope Frame IX83P2ZF	Motorized light path selection 1 100% for observation light path 2 50% for observation light path, 50% for left side port 3 100% for left side port Motorized 6-position revolving nosepiece (DIC slider attachable) Focusing movable range Upper side: 6.5 mm or more from the original position Lower side: 3 mm or more from the original position Original position: 1 mm above the stage surface Left side port image magnification: 1X
Illumination Column	Column for installing the lamp housing, with 30 ° tilting mechanism. Condenser holder up/down movement range: 88 mm Condenser refocusing mechanism is available. Designated bulb 12 V, 100 W long-life halogen bulb 12V100WHAL-L (PHILIPS 7724) Power supply: Control box IX3-CBH

Observation Tube	Type	U-BI90	U-TBI90	U-TR30-2/U-TR30H-2	U-TR30NIR
		Binocular	Tilting binocular	Trinocular	Trinocular
	Field No.	22			
	Tube inclination	45 °	35 ° to 85 °	30 °	30 °
	Interpupillary distance adjustment	50 to 76 mm			
	Diopter adjustment range	- 5 to + 5 diop			
	Intermediate tube	U-EPA2, GX-SPU, U-CA, etc.		IX-ATU	
	Light path selection	None		3 steps: ① BI 100% ② BI 20%, Camera 80% ③ Camera 100%	3 steps: ① BI 100% ② BI 50%, Camera 50% ③ Camera 100%

Stage	Type	IX3-SVR	IX2-SP
	Size	240 mm (D) x 444.5 mm (W)	240 mm (D) x 232 mm (W)
	Movement mechanism	· X- and Y-axis knob with adjustable tension. · Right long axis handle (can be mounted by reversing the right and left)	Not available
		Movement range: 75 mm vertical (Y), 114 mm horizontal (X).	Movement range: (Combined with IX-MVR) 85 mm vertical (Y), 130 mm horizontal (X).
	Option	Replaceable center plate (Ø110 mm)	Replaceable center plate (Ø110 mm)

Condenser	Type	IX3-LWUCDA	IX2-LWUCD	IX2-MLWCD	IX-ULWCD	U-UCD8 (Used together with IX-ADUCD)
	NA	0.55	0.55	0.5	0.3	When a dry top lens is used : 0.9 When an oil top lens is used : 1.4
	WD	27 mm	27 mm	45 mm	73 mm	When a dry top lens is used : 1.5 mm When an oil top lens is used : 0.6 mm
	Turret	small hole 3, large hole 4	small hole 3, large hole 2	4	4	small hole 3, large hole 5
Transportation/ storage environment	• Temperature: Min. -25 °C, Max. 65 °C • Humidity: Min. 10%, Max. 90% (without condensation)					

Item	Specification
Fluorescence illuminator IX3-RFA L-shape fluorescence illuminator IX3-RFAL IX3-RFALFE	• UIS2 optical system • Projection magnification of the field iris diaphragm: 2X (FN 22) • Aperture iris diaphragm (IX3-RFAL only)
	• Slider insertion slots ND filter slider
	• Applicable microscope: IX83P2ZF
Motorized fluorescent mirror turret IX3-RFACA Coded fluorescent mirror turret IX3-RFACS	IX3-RFACA • Observation switching: Motorized mirror unit turret (switching time approx. 0.5 sec.). • Number of mirror unit positions: 8. • Motorized shutter (Switching time: 0.2 sec.) IX3-RFACS • Observation switching: Manual mirror unit turret • Number of mirror unit positions: 8. • Manual shutter
	• Applicable microscope: IX83P2ZF,
Light source unit	Light source : U-HGLGPS
Mercury lamp housing	• 100 W Mercury lamp housing : U-LH100HG • 100 W Mercury apo lamp housing : U-LH100HGAPO • Designated bulb : 100 W Mercury burner USH-103OL Power supply: Power supply unit U-RFL-T
Xenon apo lamp housing	• 75 W Xenon apo lamp housing : U-LH75XEAPO • Designated bulb : 75 W Xenon burner UXL-75XB Power supply: Power supply unit U-RX-T

Item	Specification
Z drift compensating microscope IX3-ZDC	• Applicable microscope frame IX83P2ZF • Applicable observation tubes U-BI90, U-TBI90, U-TR30H-2*, U-TR30NIR*, U-TR30-2* * : in combination with the IX-ATU • Applicable control box IX3-CBH • Controller U-MCZ Touch panel controller PC (software “cellSens” is required to be installed in the PC.) • Laser Laser diode Laser wavelength: 790 nm, (Class 1 IEC60825-1:2007) Laser pulse duration: 2.5 ms, Repetition rate: 100 Hz Accessible laser emission (Maximum instantaneous power): 40 μW Output from laser diode: Beam divergence: 0.1 to 0.49 rad Maximum power: 20 mW • Observation method Reflected light fluorescence observation, phase contrast observation, TIRF, DIC (applicable only with focus search) • Field number 22 • Dimensions & weight 266(W) x 63(H) x 243(D) mm, approx. 2.1 kg
Operating environment	• Indoor use. • Altitude: Max. 2,000 meters • Ambient temperature: 5 to 40 °C (41 to 104° F) • Maximum relative humidity: 80 % for temperatures up to 31 °C (88 °F) (without condensation) In case of over 31 °C (88 °F), the relative humidity is decreased linearly through 70 % at 34 °C (93 °F), 60 % at 37 °C (99 °F), and to 50 % at 40 °C (104 °F). • Supply voltage fluctuations: Not to exceed ±10% of the normal voltage. • Pollution degree: 2 (in accordance with IEC60664-1) • Installation/Overvoltage category: II (in accordance with IEC60664-1)

III. TROUBLE Q&A

On This Volume

This volume describes how to deal with troubles with the FLUOVIEW FV1200 system. If any irregularity is observed, read this volume before calling for service. If the irregularity cannot be resolved by the described remedial action, please contact Olympus for repair.

1 Troubleshooting Guide

1-1 FV1200

The system may be unable to manifest its full performance due to its usage as well as malfunction. In case a problem occurs with the system please check the following list to find appropriate countermeasures.

If the problem cannot be resolved by the described remedial action, please contact Olympus for repair.

Irregularity	Cause	Remedy
1. Laser is not output from the extremity of the objective.	The laser unit is not turned ON.	Turn on the laser unit. Make sure that the emission key is set to ON.
	The laser wavelength is not selected.	Check the laser wavelength to be used.
	The manual shutter of the fluorescence mirror unit is closed. (Manual system only)	Open the manual shutter.
	The reflective mirror inside the fluorescence mirror unit is not in the light path. (Manual system only)	Engage the reflective mirror in the light path.
	The objective is not in the light path.	Engage the desired objective in the light path. When using a manual revolving nosepiece, be sure to stop the objective in the click position.
	The laser beam is too weak.	Increase the laser intensity.
	The properties of the combined cube unit used for the ASU (auxiliary scan unit) do not match the selected laser wavelength.	Engage a DM cube unit matching the selected laser wavelength in the light path.
2. Fluorescence image cannot be observed.	The confocal pinhole diameter is too small.	Increase the pinhole diameter.
	The excitation Dichroic Mirror selection does not match the observed fluorescence wavelength and excitation laser wavelength.	Engage a DM optimum for the observed fluorescence and excitation laser wavelengths.
	The spectral dichroic mirror and barrier filter selections do not match the observed fluorescence wavelength.	Engage a spectral DM and barrier filter matching the observed fluorescence in the light path.
	The acquisition wavelength region setting is not suitable for the observed fluorescence wavelength. (Spectral detection system only)	Set an acquisition wavelength region matching the observed fluorescence.
	The fluorescent dyeing method and excitation wavelength do not match each other.	Select a laser optimum for the fluorescent dyeing method.
	Focus is not adjusted.	Adjust the focus.
	The PMT voltage of the detector is too low.	Increase the PMT voltage.
2. Fluorescence image cannot be observed.	The offset value is too large.	Decrease the offset value.
	The detector for the channel to be detected is not selected.	Select the detector.

Irregularity	Cause	Remedy
3. Transmitted image cannot be observed.	The transmitted light detection channel is not selected.	Select the transmitted light detection channel.
	The transmitted light filter for the microscope is in the light path.	Disengage the filter from the light path.
	The PMT voltage of the transmitted light detection channel is too low.	Increase the PMT voltage.
	The offset value for the transmitted light detection channel is too large.	Decrease the offset value.
4. Image is disturbed.	The system installation location is subject to excessive vibrations.	Contact Olympus.
	Extraneous light such as the light of a fluorescent lamp is detected.	Turn the room light low before acquiring image.
5. Reflected light (laser light) enters the fluorescence image.	The barrier filter is set erroneously or absent.	Engage a barrier filter that can cut the excitation laser wavelength in the light path.
	The set acquisition wavelength is overlapped with or too close to the excitation laser wavelength. (Spectral detection system only)	Select an acquisition wavelength that is not interfered with by the laser wavelength. (Note that, when the confocal pinhole is large and the BS20/80 excitation DM is used, penetration of laser light may become large.)
	The barrier filter that can cut the wavelength of the laser light irradiated from the ASU (auxiliary scan unit) is not selected. In the case of a spectral detection system, the acquisition wavelength setting may be inappropriate.	Engage a barrier filter that can cut the laser wavelength from the ASU in the light path. With a spectral detection system, change the acquisition wavelength setting.
6. Fluorescence image is poor.	The front lens of the objective is dirty.	Clean the objective front lens by wiping it with a piece of gauze.
	When an objective with correction collar is in use, the correction collar is adjusted improperly.	Adjust the correction collar properly.
	The cover glass thickness is inappropriate.	Use a cover glass with thickness of 0.17 mm.
7. Fluorescence image is dark and noisy.	The laser beam is too weak.	Increase the laser intensity.
	The fluorescent dyeing method and excitation wavelength do not match each other.	Select a laser optimum for the fluorescent dyeing method.
7. Fluorescence image is dark and noisy.	The excitation Dichroic Mirror selection does not match the observed fluorescence wavelength and excitation laser wavelength.	Engage a DM optimum for the observed fluorescence and excitation laser wavelengths.

Irregularity	Cause	Remedy
	The spectral dichroic mirror and barrier filter selections do not match the observed fluorescence wavelength.	Engage a spectral DM and barrier filter matching the observed fluorescence in the light path.
	The acquisition wavelength region setting is not suitable for the observed fluorescence wavelength. (Spectral detection system only)	Set an acquisition wavelength region matching the observed fluorescence.
	The confocal pinhole diameter is too small.	Increase the pinhole diameter.
	The scanning rate is too high.	Decrease the scanning rate.
	The HV setting is too high.	Decrease the HV and increase the gain. An alternative remedy is to decrease the scanning rate and decrease the HV.
	The width of the acquisition wavelength region is too small.	Increase the width of the acquisition wavelength region.
	Dyeing is too pale.	Perform optimum fluorescent dyeing.
8. Image is irregularly blurred or the brightness is uneven.	The specimen or stage is tilted.	Install the specimen and stage properly.
9. Observed image is out of focus.	The focus is adjusted improperly.	Adjust the focus in visual observation.
10. The intensity of part of the wavelength region of the spectral characteristic data of fluorescence is dropped.	The spectral characteristics of the fluorescence are affected by those of the excitation dichroic mirror used in double excitation.	Use an excitation DM that does not affect the spectral characteristic data of fluorescence.
11. Flare is observed.	The glass in use is not fluorescence-free glass.	Use fluorescence-free glass.
	The specimen is overstained.	Perform optimum dyeing again or increase the offset value.
12. Visual fluorescent light observation is impossible.	The light path selector in the SU is not set for the visual observation light path.	Select the visual observation light path
	The light path selector in the SU is not set for the visual observation light path	Open the shutter for the mercury burner.
	The mirror unit incorporating a dichroic mirror is not present in the turret of the illuminator	Engage a mirror unit containing DM in the light path.
13. The light from the laser for the ASU (additional scan unit) is not output.	The properties of the combined DM cube unit for ASU do not match the irradiated laser wavelength	Engage a DM cube unit matching the laser wavelength in the light path
	The combined DM cube unit for ASU is not in the light path.	Engage the DM cube unit in the light path.

1-2 IX83P2ZF

Problem	Cause	Remedy
a) The bulb does not light.	Power cord of the IX3-CBH is unplugged.	Plug the power cord into a power outlet.
	Main switch of the IX3-CBH is not " I " ON.	Set the main switch to " I " (ON).
	Bulb is burnt out.	Replace the bulb.
b) The bulb lights but the field of view is dark.	Lamp voltage is too low.	Increase the light intensity to an optimum voltage.
	Condenser is not well positioned.	Adjust the condenser height until the field iris diaphragm image is formed in the specimen plane.
	Light path selector knob is set for the left side port light path.	Change the light path.
	Too many filters are used.	Reduce the number of filters to the minimum required.
	Stage center plate is engaged in the light path.	Move the stage and place the specimen again.
	Field iris diaphragm is not opened wide enough.	Open the field iris diaphragm sufficiently.
c) Field of view is obscured or not evenly illuminated.	An objective that falls outside the condenser's illumination range is used.	Use a condenser that matches the objective.
	Field iris diaphragm is not properly centered.	Center the field iris diaphragm correctly
	Field iris diaphragm is stopped down too far.	Open the field iris diaphragm sufficiently.
	A filter is stopped in an intermediate position.	Set the filter at the appropriate position.
d) Dirt or dust is visible in the field of view.	Dirt/dust on the specimen.	Clean thoroughly.
	Dirt/dust on the eyepieces.	
	Dirt/dust on a mirror unit.	
	Dirt/dust on the optical element.	
	Condenser is not correctly positioned and the frost filter Ø45 mm (45FR) or filter is focused.	Adjust the condenser height until the field iris diaphragm image is formed in the specimen plane.
e) Image glares.	Condenser is raised too high.	Lower to the proper position
	Aperture iris diaphragm is stopped down too far.	Open the aperture iris diaphragm.

Problem	Cause	Remedy
f) Error happens at the time of system starting-up.	There are some mistakes in the order of turning on the power switches.	Turn off all of the power switches, then turn on the switches again in the correct order. As to the correct order in turning on the power switches, refer to the page 12.
g) Visibility of observed image is poor. • Image is not sharp. • Contrast is poor. • Details are poorly visible.	Objective in use is not designed for UIS2 series.	Replace with an objective designed for UIS2 optics.
	Correction collar on the objective equipped with correction collar is not adjusted.	Adjust the correction collar to acquire the best contrast.
	Front lens of the objective is dirty.	Clean the objective.
	The immersion oil appropriate with an oil immersion objective is not used.	Use Olympus immersion oil with the oil immersion objective, Olympus silicone oil with the silicone immersion objective and water with the water immersion objective.
	Immersion oil contains bubbles.	Remove bubbles.
	Inappropriate slide or cover glass thickness.	Replace with glass of appropriate thickness.
	Glass components (condenser, objective, eyepieces, culture vessels, etc.) are dirty.	Clean thoroughly.
	Ring slit and phase plate are not centered.	Center them correctly.
	A plastic culture vessel is used.	Replace the plastic culture vessel with a glass vessel.
h) A part or one side of the field of view is blurred.	Specimen is tilted with respect to the stage.	Place the specimen correctly on the stage and secure it with the stage clip.
i) Field of view of one eye does not match that of the other.	The interpupillary distance is incorrect.	Adjust the interpupillary distance.
	Incorrect diopter adjustment.	Adjust the diopter.
	You are not accustomed to parallel optical axis.	When looking into the eyepieces, do not stare at image from the beginning but see the overall field of view. It is sometimes recommended to turn your eyes away from the eyepieces, look far off and look into the eyepieces again.

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OLYMPUS®

User’s Manual

[Quick Start]

FLUOVIEW
FV1200MPE
FV1200

Multi Photon Laser Scanning Microscope
Biological Confocal Laser Scanning Microscope

FV10-ASW

Notice

Thank you for your purchase of Olympus microscope at this time.
Retain this manual in an easily accessible place near a system for future reference.

AX8268

Caution

FV1200MPE is a CLASS 4 laser product; FV1200 is a CLASS 3B laser product.

The procedures for using this system are classified as follows:

- Service

  “Service” means any adjustment or repair performed by service personnels who are provided the service training following to the service manual for this system.

  The performance has influence on the feature of this system, and there is a risk which unintended CLASS 3B or CLASS 4 laser light is emitted.

- Maintenance

  “Maintenance” means adjustment or other procedures performed by customers to maintain that this system functions properly.

- Operation

  “Operation” means all performance described in the user’s manuals in this system.

  CLASS 3B or CLASS 4 laser light is only emitted from the objective lens during the actual execution.

The User’s Manuals of this system consist of the following:

In order to maintain the full performance of this system and ensure your safety, be sure to read these user’s manuals and the operating instructions for the laser unit and light source unit before use.

	User’s Manual Sets	
	FV1200MPE	FV1200
FV1200MPE / FV1200 User’s Manual [Laser Safety Guide]	○	○
FV1200MPE User’s Manual [Safety Manual]	○	
FV1200 User’s Manual [Safety Manual]	○	○
FV1200MPE User’s Manual [Operation / Maintenance]	○	
FV1200 User’s Manual [Operation / Maintenance]	○	○
FV1200 FV10-ASW User’s Manual [Quick Start]	○	○

Also, we have prepared one service manual for this system as below. Technical personnels who perform the service are required to take the service training.

- FV1200MPE / FV1200 Service Manual

In case of purchasing the laser simultaneously, we have prepared the following manual for the laser.

- MaiTai Series User’s Manual [Quick Start]
- Chameleon User’s Manual [Quick Start]

In addition, we have prepared one service manual for the laser as below. Technical personnels who perform the laser service require to take the service training.

- MaiTai Series Service Manual
- Chameleon Service Manual

Part or whole of this software as well as manual shall not be used or duplicated without consent.

For customers using FV1000MPE or FV1000:

This manual contains the instructions for FV1200MPE / FV1200 and FV-10ASW (Ver.4.0 and later version).

If the instructions for FV1000MPE / FV1000 and (former version than Ver.4.0) are not covered by this manual,refrer to the manual for FV1000MPE / FV1000 on your hand.

Registered trademark

Microsoft, Microsoft Windows are registered trademarks of Microsoft Corporation.

Other brand names and product names are trademarks or registered trademarks of their respective owners.

This Quick Start has divided into the volume by the following system configurations.

IX83

IX81

BX61

BX61WI

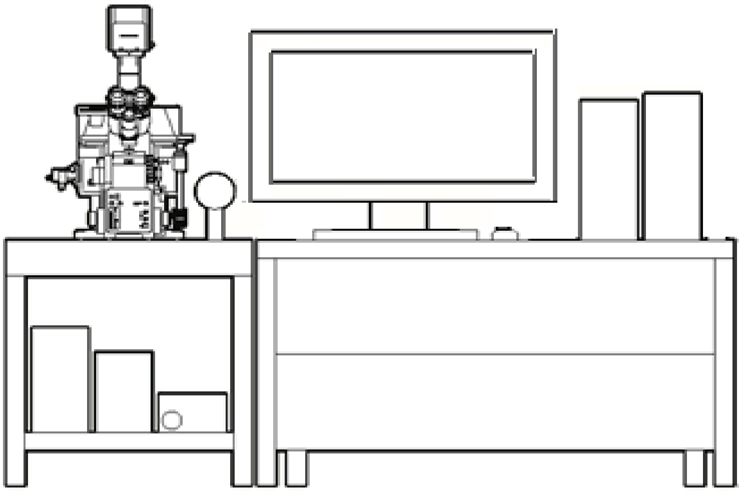
OLYMPUS®

Laser Scanning Biological Microscope

FV1200

IX83

Quick Start



FV1200 IX83
Quick Start
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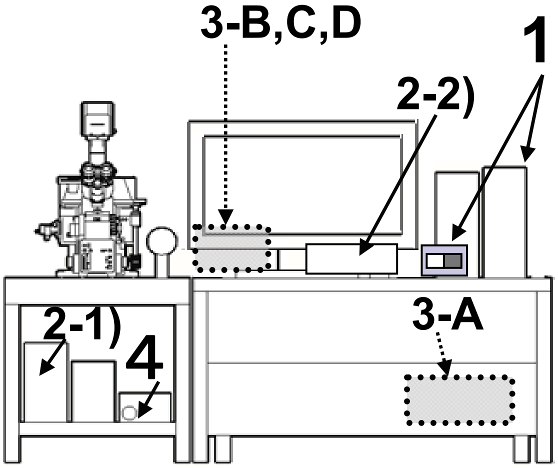
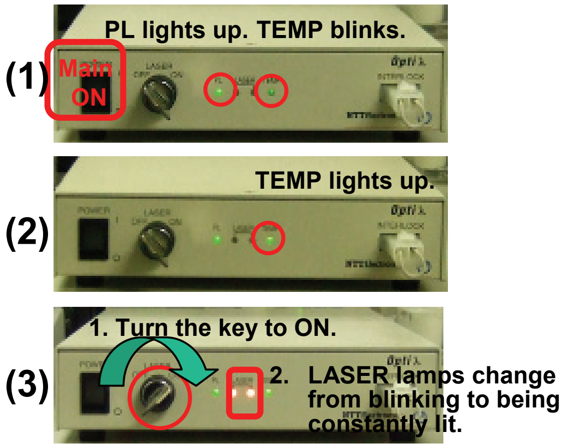
Image Acquisition

— Fundamentals —

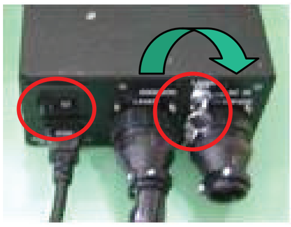
System Startup


3-B,C,D
1
2-2)
2-1)
4
3-A

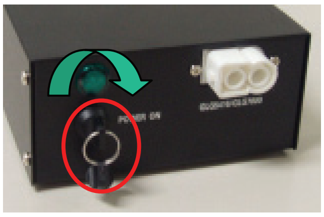
B: LD559 laser


PL lights up. TEMP blinks.
(1)
Main ON
(2)
TEMP lights up.
(3)
1. Turn the key to ON.
2. LASER lamps change from blinking to being constantly lit.

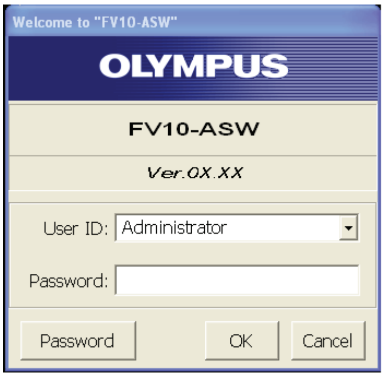
C: MAr laser



D: HeNe (G) laser



5


Welcome to "FV10-ASW"

OLYMPUS

FV10-ASW

Ver.0X.XX

User ID: Administrator

Password:

Password
OK
Cancel

1. Switch on the central power and then start up the Controller.
2. Set the power switches of the units of microscope to I(ON) according to the following order:
   1) IX3-CBH
   2)Touch panel controller
3. Start up the laser systems.

A: MCPSU (405/440/473/559/635 nm)

Switch on the system.

B: LD559

(1)Switch on the main power of the laser system. The PL lamp lights up in **green** and the TEMP lamp blinks in **green**.
(2)Confirm that the TEMP lamp has changed from blinking to being constantly lit.
(3)Turn on the key switch. The LASER lamps **blink in red**. It takes a while for the lasers to stabilize (The lamps change from **blinking in red** to being constantly **lit in red**). After the LASER lamps are **lit in red**, the LD559 laser system is ready for use.

C: MultiAr laser

Switch on the main power and then turn the key switch clockwise.

D: HeNe (G) laser (543 nm)

Turn the key switch clockwise.

3. Switch on the power of the mercury lamp unit. *** Used for visual observation**
4. Enter the user name and password to log on to Windows.

OS	User name	Passwprd
Windows7 (64bit)	olympus	olympus
Others	fluoview	fluoview

5. Double click FV to start up the FV10-ASW software.

User name: Administrator
Password: Administrator

Visual Observation via Microscope

■ ■ Fluorescent image observation ■ ■

■ Memo ■

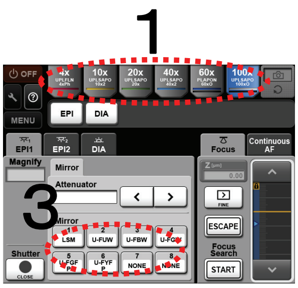
Fluorescent mirror unit

WBV: blue violet excitation (ECFP, etc.)

NIBA: blue excitation/green fluorescence (Example: FITC, EGFP, etc.)

WIG: green excitation/red fluorescence (Example: rhodamine, DsRed, etc.)

WU: UV excitation


1
OFF
4x
10x
20x
40x
60x
100x
EPI
DIA
MENU
EPI1
EPI2
DIA
Focus
Continuous AF
Magnify
Mirror
Attenuator
3
Mirror
LSM
U-FUW
U-FBW
U-FGW
U-FGF
U-FYF
NONE
NONE
Shutter
CLOSE
ESCAPE
Focus Search
START

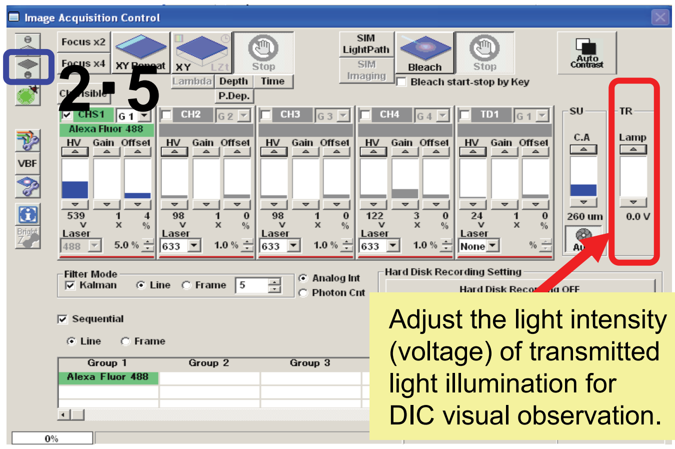
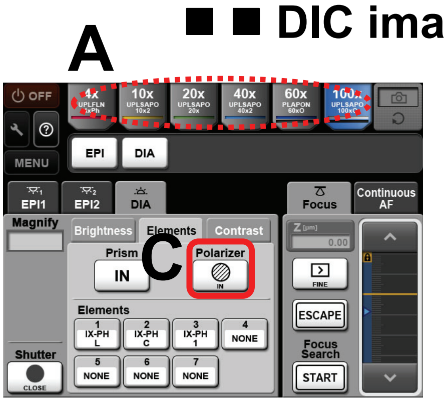


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
P.Dep.
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
2·5
CHS1
CH2
CH3
CH4
TD1
Alexa Fluor 488
HV Gain Offset
Laser
SU
TR
C.A
Lamp
260 um
0.0 V
VBF
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Hard Disk Recording Setting
Hard Disk Recording OFF
Sequential
Line
Frame
Group 1
Group 2
Group 3
Alexa Fluor 488

Adjust the light intensity (voltage) of transmitted light illumination for DIC visual observation.

1. Select an objective lens using the touch panel controller.
2. Click in the FV10-ASW software.
3. Select a fluorescent mirror unit using the touch panel controller.
4. Adjust the focus.
5. After observation finishes, click to close the shutter of the fluorescent lamp.

■ ■ DIC image observation ■ ■


A
OFF
4x
10x
20x
40x
60x
100x
EPI
DIA
MENU
EPI1
EPI2
DIA
Focus
Continuous AF
Magnify
Brightness
Elements
Contrast
Prism
IN
C
Polarizer
IN
Elements
IX-PH L
IX-PH C
IX-PH 1
NONE
NONE
NONE
NONE
Shutter
CLOSE
FINE
ESCAPE
Focus Search
START

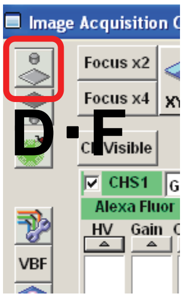
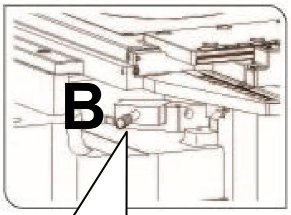


Image Acquisition C
Focus x2
Focus x4
D·F
Visible
CHS1
Alexa Fluor
HV
Gain
VBF



Adjust the DIC contrast using this control.

A. Select an objective lens using the touch panel controller.

B. Insert the DIC slider.

C. Insert the polarizer using the touch panel controller.

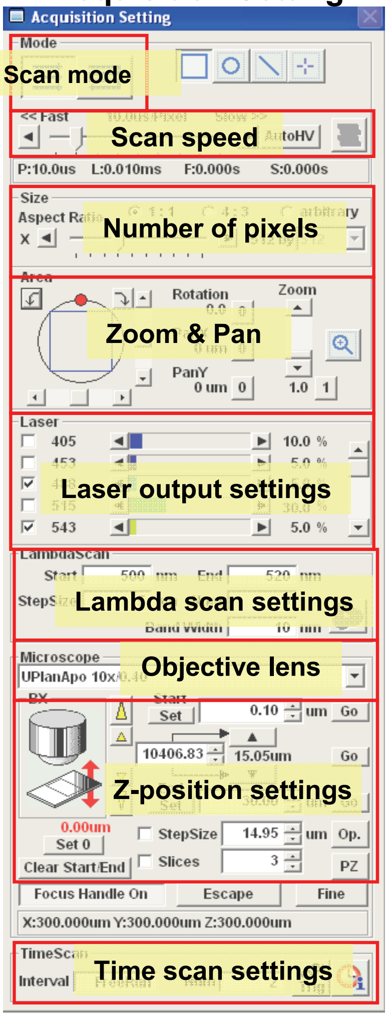
D. Click in the FV10-ASW software.

E. Adjust the focus.

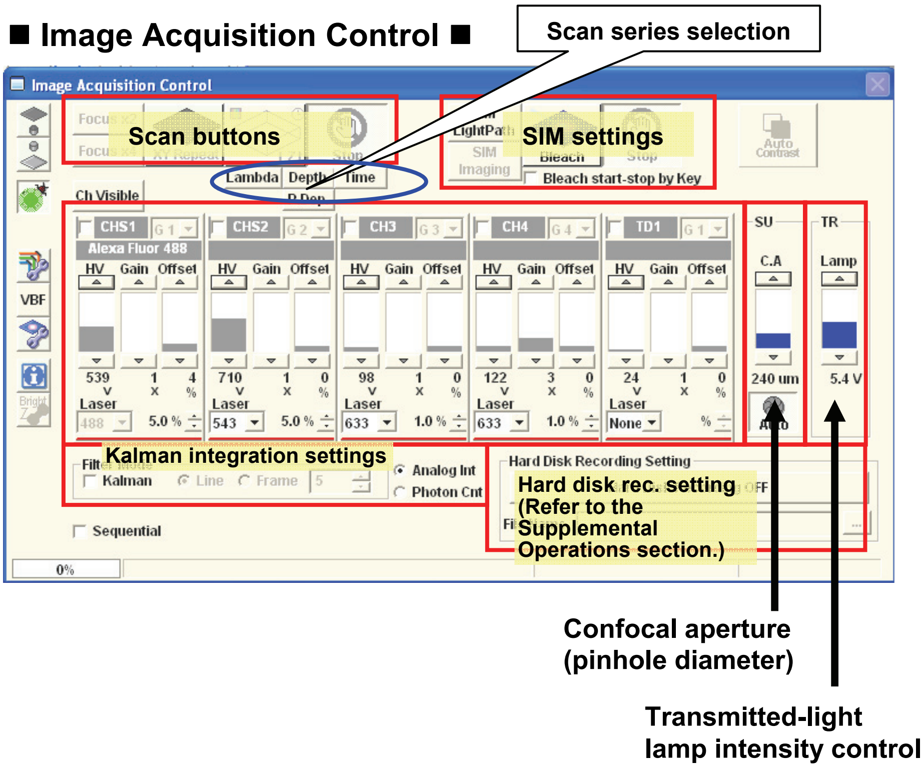
F. After observation finishes, click to close the shutter of the halogen lamp.

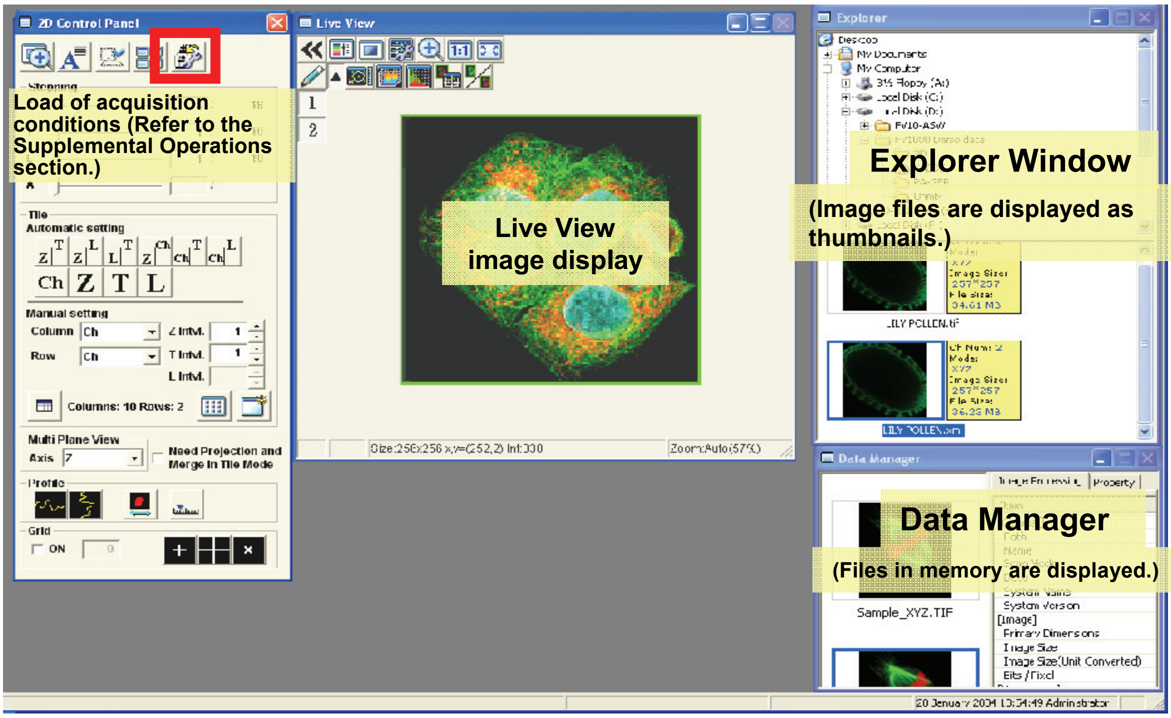
Overview of Image Acquisition Panel

■ Acquisition Setting ■


Acquisition Setting
Mode
Scan mode
<< Fast
Scan speed
AutoHV
P:10.0us L:0.010ms F:0.000s S:0.000s
Size
Aspect Ratio
Number of pixels
Area
Rotation
Zoom
Zoom & Pan
PanY
0 um
1.0
Laser
405 10.0 %
Laser output settings
543 5.0 %
LambdaScan
Lambda scan settings
Microscope
UPlanApo 10x
Objective lens
Set
0.10 um Go
10406.83
15.05um
Z-position settings
0.00um
Set 0
StepSize 14.95 um Op.
Clear Start/End
Slices 3 PZ
Focus Handle On
Escape
Fine
X:300.000um Y:300.000um Z:300.000um
TimeScan
Interval
Time scan settings

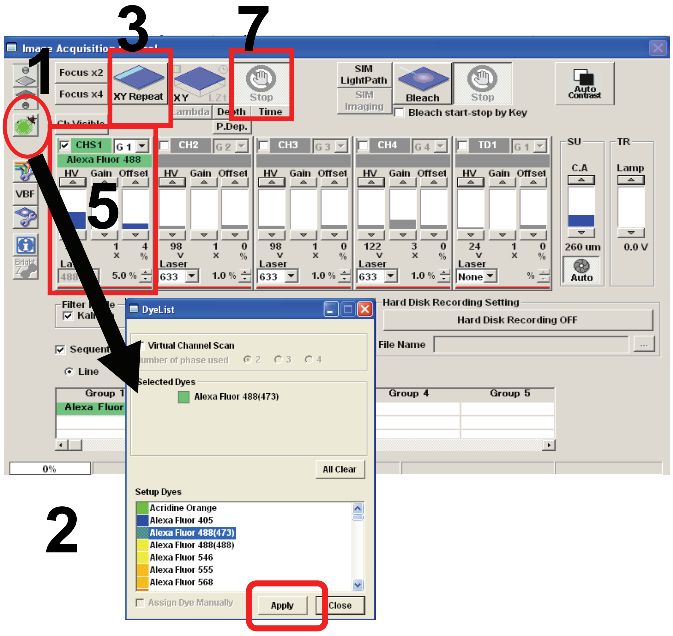
■ Image Acquisition Control ■


Scan series selection
Image Acquisition Control
Scan buttons
Lambda
Depth
Time
SIM settings
LightPath
SIM Imaging
Bleach
Bleach start-stop by Key
Auto Contrast
Ch Visible
VBF
CHS1
Alexa Fluor 488
CHS2
CH3
CH4
TD1
HV Gain Offset
539
710
98
122
24
Laser
488
543
633
633
None
5.0 %
1.0 %
SU
C.A
240 um
Auto
TR
Lamp
5.4 V
Kalman integration settings
Kalman
Line
Frame
Analog Int
Photon Cnt
Hard Disk Recording Setting
Hard disk rec. setting (Refer to the Supplemental Operations section.)
Sequential
0%
Confocal aperture (pinhole diameter)
Transmitted-light lamp intensity control

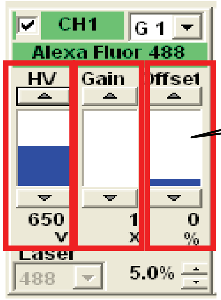

2D Control Panel
Load of acquisition conditions (Refer to the Supplemental Operations section.)
Tile
Automatic setting
Ch Z T L
Manual setting
Column
Row
Z Intvl.
T Intvl.
L Intvl.
Columns: 10 Rows: 2
Multi Plane View
Axis
Need Projection and Merge In Tile Mode
Profile
Grid
ON
Live View
Live View image display
Size:256x256 x,y=(252,2) Int:330
Zoom:Auto(57%)
Explorer
Explorer Window
(Image files are displayed as thumbnails.)
LILY POLLEN.tif
Data Manager
Data Manager
(Files in memory are displayed.)
Sample_XYZ.TIF
Property
Image Size
Image Size(Unit Converted)
Bits /Pixel

XY Image Acquisition (Single-color staining)

Example: single-color staining with green fluorescence (Alexa488)


1
3
7
5
2
Focus x2
Focus x4
XY Repeat
Stop
Time
Alexa Fluor 488
DyeList
Selected Dyes
Alexa Fluor 488(473)
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Apply
Close

5


CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650
1
0
Laser
488
5.0%

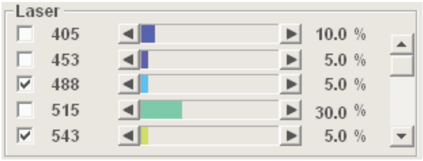
Ch group box adjustment

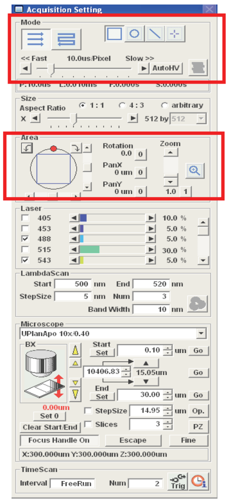
HV: Adjusts detector sensitivity.

Gain: Brightens images with a set magnification.

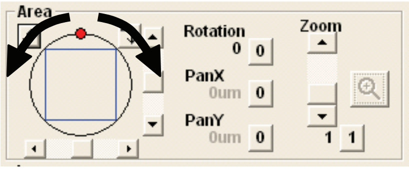
Offset: Darkens the background.

Laser intensity adjustment

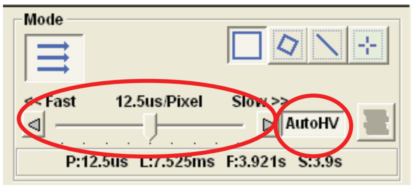

Laser
405
10.0 %
453
5.0 %
488
5.0 %
515
30.0 %
543
5.0 %


Acquisition Setting
Mode
Area
Rotation
Zoom
Laser
Microscope

6 Rotation Zoom


Area
Rotation
PanX
PanY
Zoom

8 Rotation


Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us L:7.525ms F:3.921s S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Click the XY Repeat button XY Repeat to execute a continuous scan.

* If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4 .

4. Adjust the focus by turning the focus knob.

5. Make adjustments for green imaging (Alexa488) (laser intensity, HV, offset, etc.).

6. Set the zoom and rotation as required.

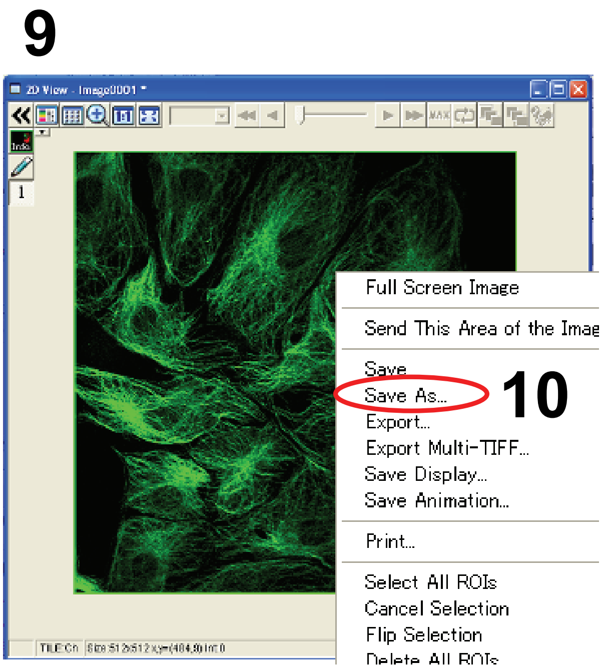
7. After finishing image adjustment, click the STOP button Stop to stop scanning.

8. Select AutoHV and then select ScanSpeed.

* The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
Alternatively, Kalman integration can be used.
For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Single-color staining)

Example: single-color staining with green fluorescence (Alexa488)


9
2D View - Image0001
Full Screen Image
Send This Area of the Imag
Save
Save As...
10
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

9. To acquire the image, click the XY button.
10. Save the image: Select **Save As** by right-clicking or going to the File menu, and then save the image in OIB or OIF format.

> **■ Memo ■**
> **FV10-ASW dedicated file formats**
>
> OIF format:
> Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.
>
> OIB format:
> In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an image as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

[Points on acquiring clear images]

For dark images

Increasing the HV: Increases detector sensitivity. Use another method in addition to this if sufficient brightness cannot be obtained even when the HV is increased to 700 V or higher.

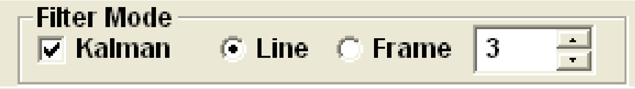
Increasing laser intensity: Increases fluorescent brightness with an increase in excitation light output. Note that an excessively increased intensity may fade fluorescence or cause greater damage to samples.

Increasing the CA: The Auto button stays clicked during regular use so that the optimal pinhole diameter is always automatically set.
Higher brightness can be obtained by deselecting Auto to increase the CA (confocal aperture), which is equivalent to decreasing the Z-axis resolution.

Increasing the gain: Brightens images with the magnification set before image acquisition.

For noisy images

Kalman integration ... Averages images while acquiring as many images as specified. Check the checkbox for Kalman and then select Line (or Frame). Enter **the number of image integrations (the number of scans)**.

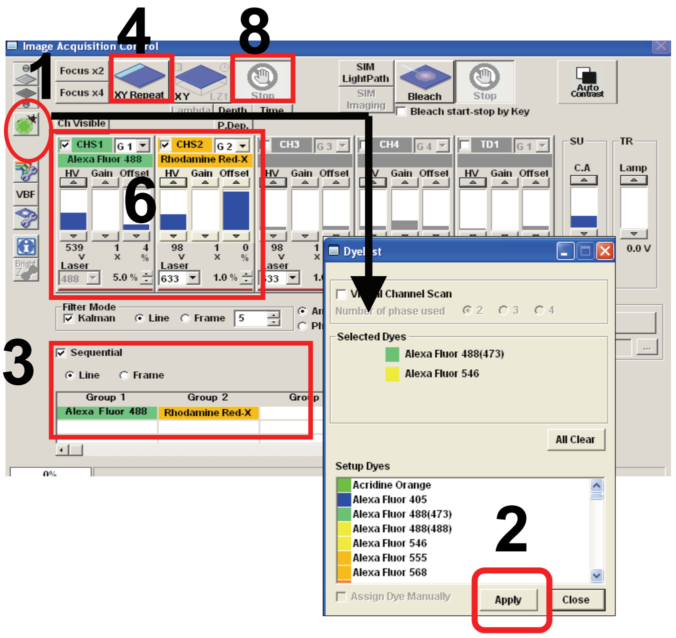


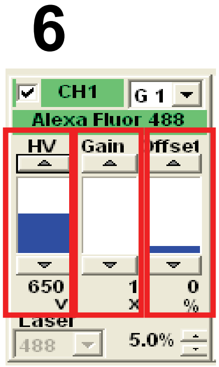
Filter Mode
☑ Kalman ● Line ○ Frame 3

XY Image Acquisition (Multicolor staining)

Line sequential scan

* Mode for avoiding fluorescent crosstalk by individually acquiring fluorescent images


1
4
8
6
3
2
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
SIM LightPath
SIM Imaging
Bleach
Bleach start-stop by Key
Auto Contrast
Ch Visible
CHS1
Alexa Fluor 488
CHS2
Rhodamine Red-X
CH3
CH4
TD1
HV
Gain
Offset
Laser
Filter Mode
Kalman
Line
Frame
Sequential
Group 1
Group 2
Dyelist
Virtual Channel Scan
Number of phase used
Selected Dyes
Alexa Fluor 488(473)
Alexa Fluor 546
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close


6
CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650
V
1
X
0
%
Laser
488
5.0%

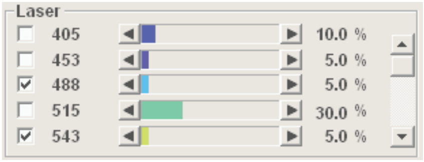
Ch group box adjustment

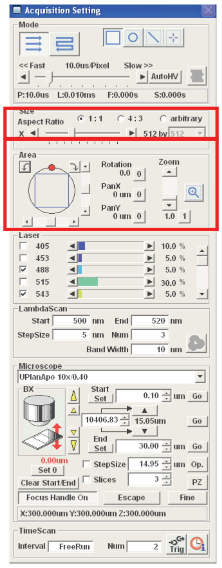
HV: Adjusts detector sensitivity.

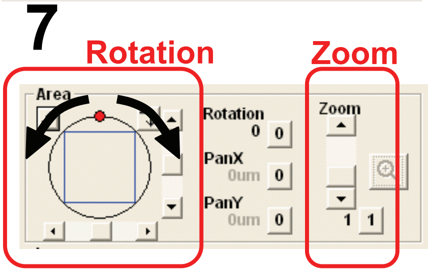
Gain: Brightens images with a set magnification.

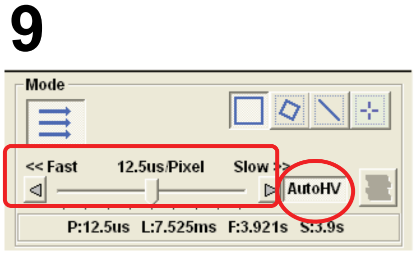
Offset: Darkens the background.

Laser intensity adjustment


Laser
405
453
488
515
543
10.0 %
5.0 %
5.0 %
30.0 %
5.0 %


Acquisition Setting
Mode
Size
Aspect Ratio
Area
Rotation
Zoom
Laser
LambdaScan
Microscope
TimeScan


7
Rotation
Zoom
Area
Rotation
PanX
PanY
0um


9
Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us
L:7.525ms
F:3.921s
S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Check the checkbox for Sequential and then select Line.

4. Click the XY Repeat button XY Repeat to execute a continuous scan.

   * If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4 .

5. Adjust the focus by turning the focus knob.

6. Make adjustments for CH1/CH2 imaging (laser intensity, HV, offset, etc.).

7. Set the zoom and rotation as required.

8. After finishing image adjustment, click the STOP button Stop to stop scanning.

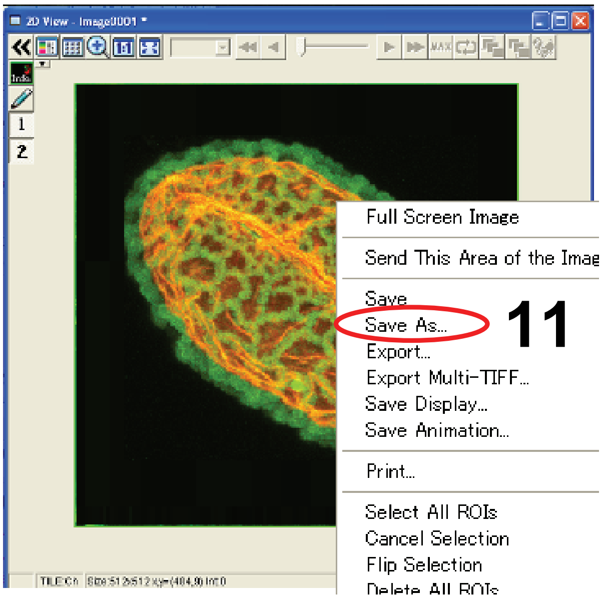
9. Select AutoHV and then select ScanSpeed.

   * The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
   Alternatively, Kalman integration can be used.
   For more information, refer to page 8, "Points on acquiring clear images."

XY Image Acquisition (Multicolor staining)

Line sequential scan

10


2D View - Image0001
1
2
Full Screen Image
Send This Area of the Imag
Save
Save As...
11
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

10. To acquire the image, click the XY button.
11. Save the image: Select **Save As** by right-clicking or going to the File menu, and then save the image in OIB or OIF format.

■ Memo ■
FV10-ASW dedicated file formats

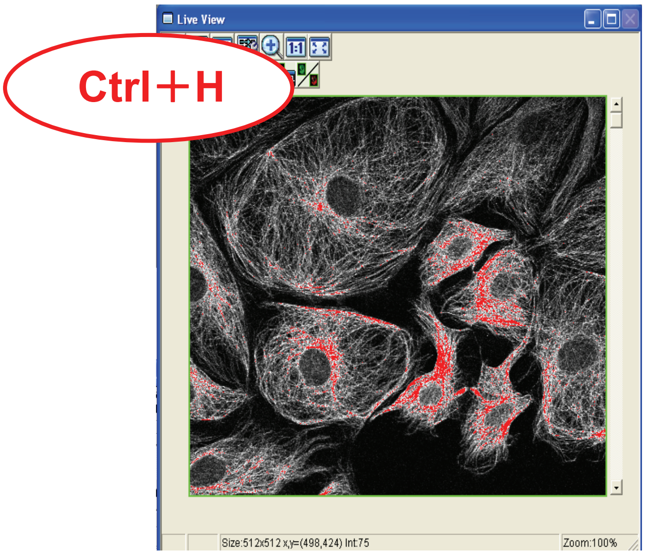
OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

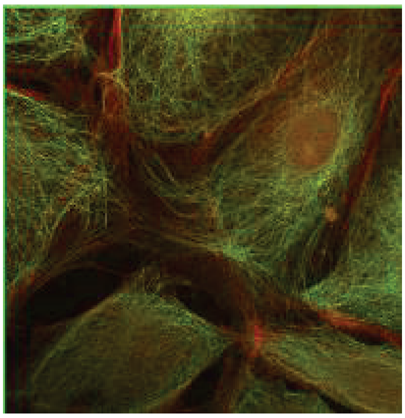
OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an image as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

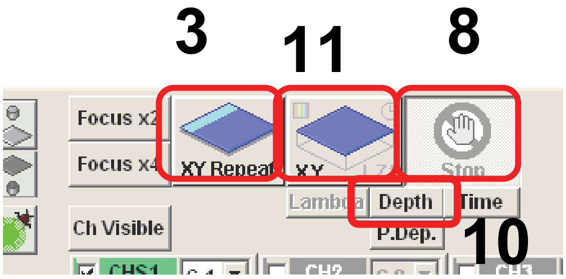
■ Image brightness adjustment ■

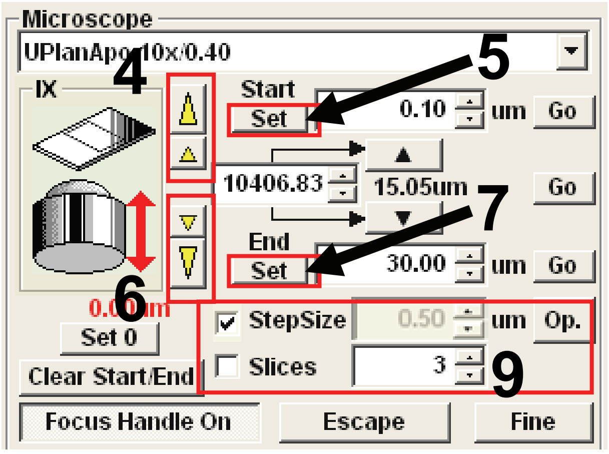
By pressing **Ctrl + H** on the keyboard, an image can be displayed in Hi-Low mode. Pixels of intensity 4095 are shown in red.
To return to normal mode from Hi-Low mode, press **Ctrl + H** again. The image returns to its original color.


Live View
Ctrl＋H
Size:512x512 x,y=(498,424) Int:75
Zoom:100%



XYZ Image Acquisition

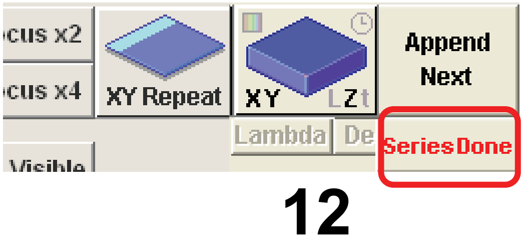

3
11
8
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
P.Dep.
10
Ch Visible
CHS1
CH2
CH3


Microscope
UPlanApo 10x/0.40
4
IX
Start
Set
0.10
um
Go
5
10406.83
15.05um
Go
7
End
Set
30.00
um
Go
6
0.00um
Set 0
☑ StepSize
0.50
um
Op.
☐ Slices
3
9
Clear Start/End
Focus Handle On
Escape
Fine

■ Memo ■

△ : Each click shifts the position by the value entered for StepSize.

△ : Each click shifts the position by 1/2 the value entered for StepSize.


cus x2
cus x4
XY Repeat
XY
LZt
Append
Next
Lambda
De
Series Done
Visible
12

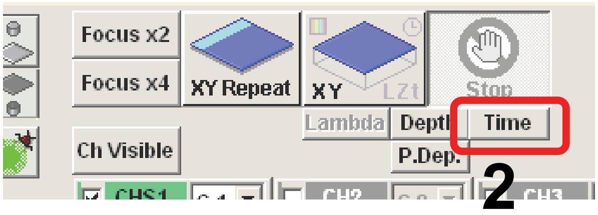
1. Before starting the following procedure, make adjustments for XY imaging.
   (Refer to page 6 or 8.)

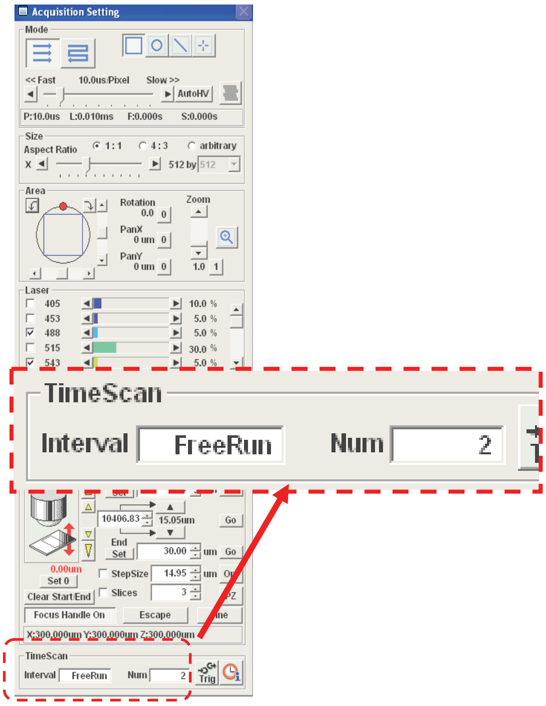
The following procedure fixes the upper and lower limit positions of a continuous cross-sectional image.

2. Click Clear Start/End to clear the settings of acquisition start and end position. Then, click Set 0 to zero the Z-position values being displayed.
3. Click the XY Repeat button to execute a scan.
4. Click △ or △ to shift the focus.
   (Refer to ■ Memo 1 ■.)
5. When the upper limit position of the sample is displayed in the image, fix the position by clicking the Set button.
6. Click ▽ or ▽ to shift the focus.
   (Refer to ■ Memo 1 ■.)
7. When the lower limit position of the sample is displayed in the image, fix the position by clicking the Set button.
8. To stop scanning, click the Stop button.
9. Enter a value in StepSize or Slices, and check ☑ the checkbox.
   (Click the Op button to see a recommended value.)
10. Select Depth.
11. To acquire the image, click the XYZ button.
12. To save the image, click Series Done.

For more information on 3D image display, refer to the Supplemental Operations section.

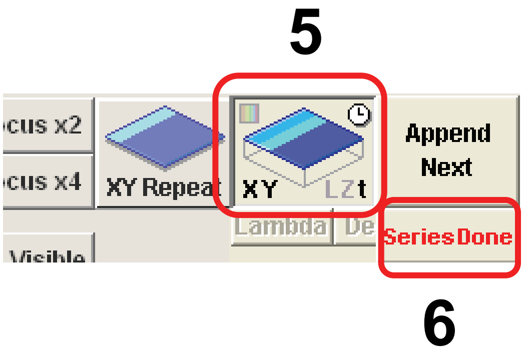
XYT Image Acquisition


Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
P.Dep.
Ch Visible
CHS1
CH2
CH3
2


Acquisition Setting
Mode
Size
Area
Laser
Trig

TimeScan

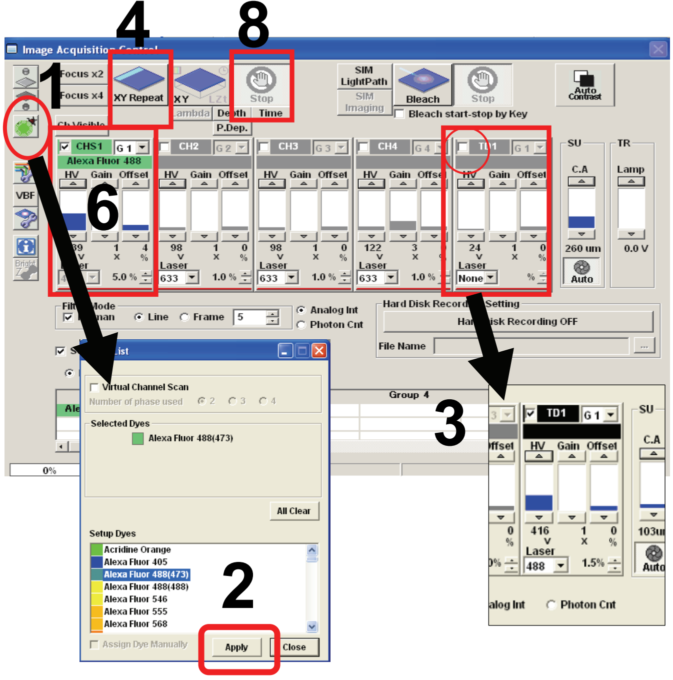
Interval FreeRun Num 2


5
Focus x2
Focus x4
XY Repeat
XY
LZt
Append Next
Lambda
Series Done
Visible
6

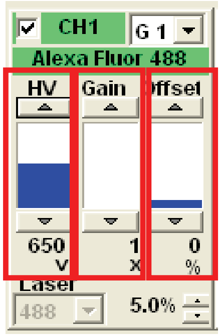
1. Before starting the following procedure, make adjustments for XY imaging.
   (Refer to page 6 or 8.)
2. Select Time from among the series buttons.
3. In the Interval box, enter the time (in seconds) taken between one acquisition start and the next acquisition start.
   Example: To set a 1 minute interval, type “60” and click Enter.
4. Enter the number of acquisition in the Num box.
5. To acquire the images, click the XYT button.
6. To save the images, click Series Done.

XY Image Acquisition (Single-color staining + DIC)

Example: green fluorescent + DIC image


4
8
1
6
3
2
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
P.Dep.
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
CH1
Alexa Fluor 488
CH2
CH3
CH4
TD1
HV Gain Offset
SU
TR
C.A
Lamp
260 um
0.0 V
Auto
Line
Frame
Analog Int
Photon Cnt
Hard Disk Recording Setting
Hard Disk Recording OFF
File Name
Group 4
Virtual Channel Scan
Number of phase used
Selected Dyes
Alexa Fluor 488(473)
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close

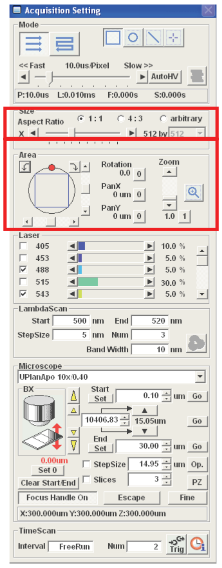
6


CH1
G1
Alexa Fluor 488
HV
Gain
Offset
650 V
1 X
0 %
Laser
488
5.0%

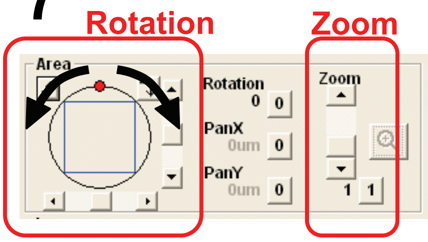
HV: Adjusts detector sensitivity.

Gain: Brightens images with a set magnification.

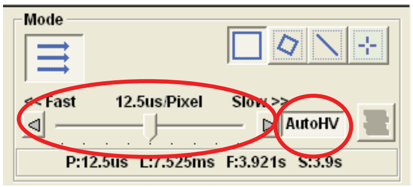
Offset: Darkens the background.


Acquisition Setting
Mode
<< Fast
10.0us/Pixel
Slow >>
AutoHV
Size
Aspect Ratio
1:1
4:3
arbitrary
Area
Rotation
PanX
PanY
Zoom
Laser
LambdaScan
Microscope
UPlanApo 10x/0.40
TimeScan

7


Rotation
Zoom
Area
Rotation
0
PanX
0um
0
PanY
0um
0
Zoom
1
1

9


Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us L:7.525ms F:3.921s S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Check the checkbox for TD1.
   Check that the polarizer and the DIC slider for transmitted light are within the optical path limits.

4. Click the XY Repeat button XY Repeat to execute a continuous scan.
   * If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4.

5. Adjust the focus by turning the focus knob.

6. Make adjustments for green imaging (Alexa488) and TD imaging.

7. Set the zoom and rotation as required.

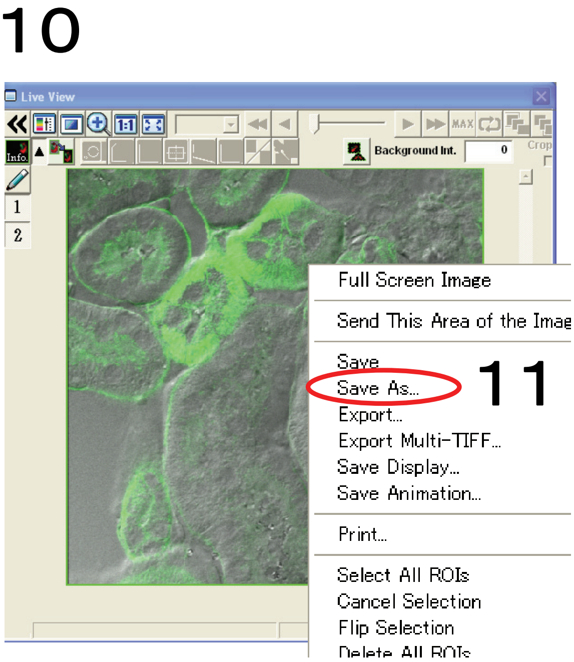
8. After finishing image adjustment, click the STOP button Stop to stop scanning.

9. Select AutoHV and then select ScanSpeed.

   * The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
   Alternatively, Kalman integration can be used.
   For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Single-color staining + DIC)

Example: green fluorescent + DIC image


10
Live View
Info.
1
2
Background Int.
0
Crop
Full Screen Image
Send This Area of the Imag
Save
Save As...
11
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

10. To acquire the image, click the XY button.

11. Save the image: Select **Save As** by right-clicking or going to the File menu and then save the image in OIB or OIF format.

■ **Memo** ■
FV10-ASW dedicated file formats

OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

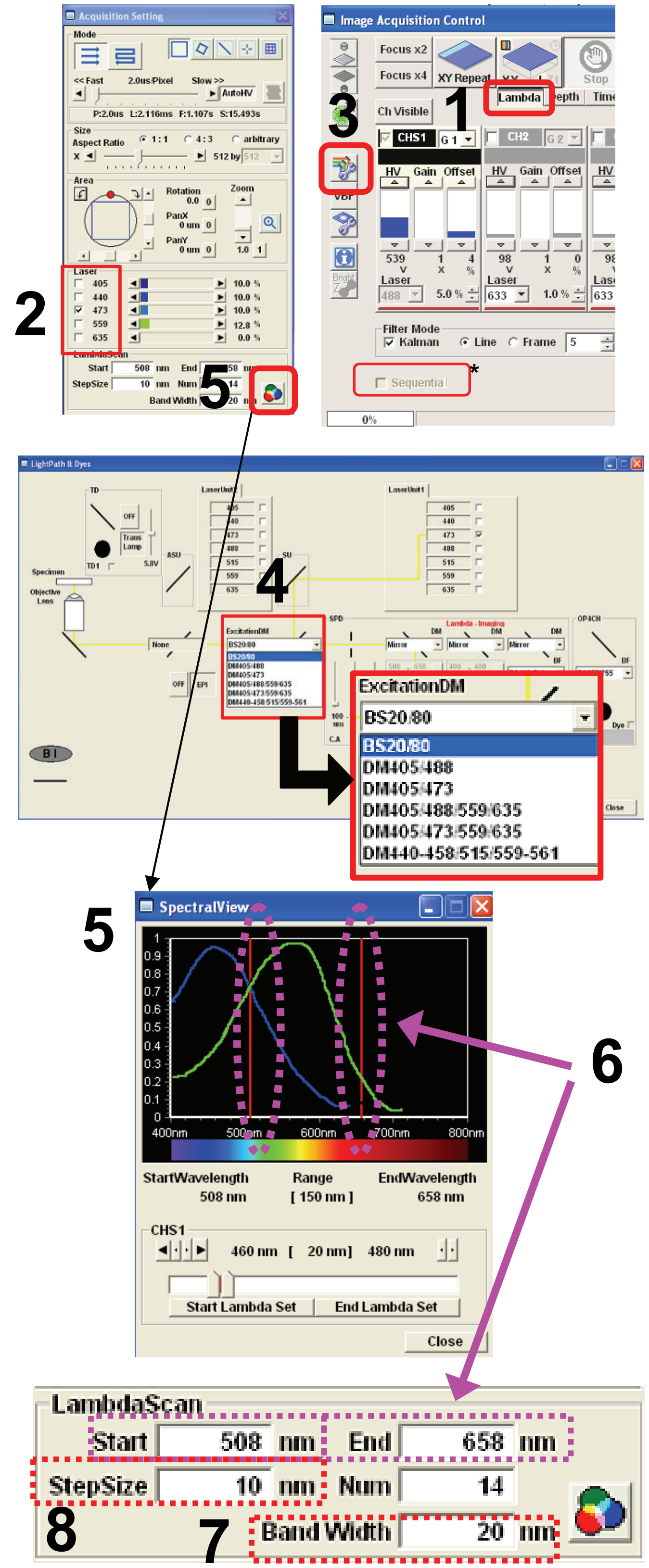
For more information on how to export an images as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

14

Only for SPD

XYλ Image Acquisition

Spectral image acquisition


Acquisition Setting
Image Acquisition Control
Lambda
Sequential
LightPath & Dyes
ExcitationDM
BS20/80
DM405/488
DM405/473
DM405/488/559/635
DM405/473/559/635
DM440-458/515/559-561
SpectralView
400nm
500nm
600nm
700nm
800nm
Start Lambda Set
End Lambda Set
Close
LambdaScan
1
2
3
4
5
5
6
7
8

StartWavelength 508 nm
Range [150 nm]
EndWavelength 658 nm

CHS1 460 nm [20 nm] 480 nm

Start 508 nm
End 658 nm
StepSize 10 nm
Num 14
Band Width 20 nm

1. Click **Lambda** .

   * When the Sequential checkbox is checked, the Lambda button is grayed out. Uncheck the checkbox.

2. Check the checkbox for the laser to be used.

3. To display the optical path diagram, click .

4. Select BS20/80 or a DM according to the excitation wavelength.

   Example: Select DM405/473 for excitation at 473.

5. To display SpectralView, click .

6. In the Start and End boxes, enter the wavelength at which to start and end image acquisition.

   These boxes correspond to the red bars in the SpectralView graph.

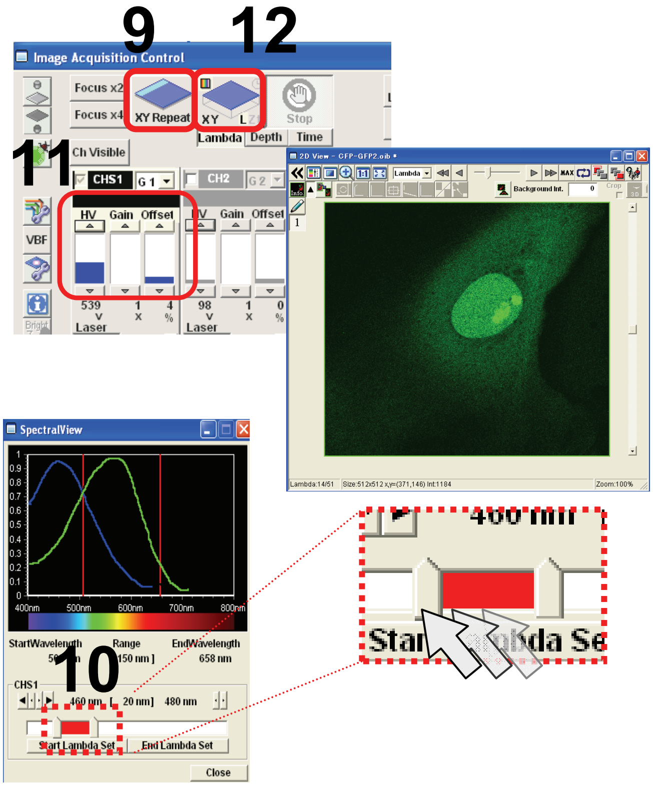
7. In the Band Width box, set the wavelength range of acquisition per frame (Example: 20 nm).

8. In the StepSize box, enter the wavelength interval between one acquisition start and the next acquisition start (Example: 10 nm).

   The minimum settable unit is 1.0 nm.

XYλ Image Acquisition

Spectral image acquisition


9
12
11
10
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
Ch Visible
CHS1
CH2
HV
Gain
Offset
VBF
Laser
2D View - GFP-GFP2.oib
SpectralView
400nm
500nm
600nm
700nm
800nm
StartWavelength
Range
EndWavelength
658 nm
460 nm
20 nm
480 nm
Start Lambda Set
End Lambda Set
Close
400 nm

9. To execute a scan, click the XY Repeat button XY Repeat .

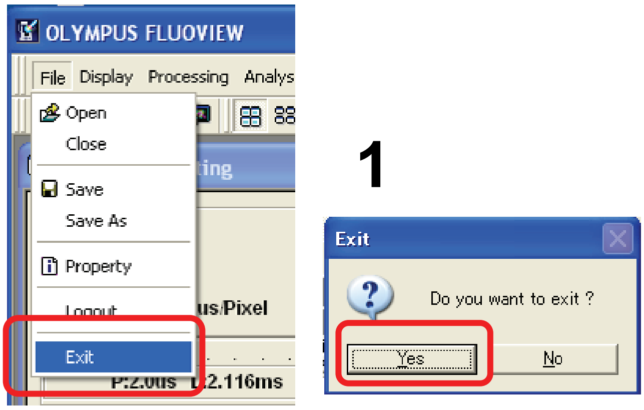
10. While observing the image, find the brightest fluorescent wavelength range by clicking the left slit.

11. Adjust the image.

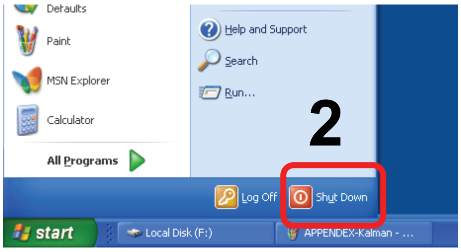
12. To acquire the image, click the XYL button XY LZt .

13. To save the λ image, click SeriesDone SeriesDone .

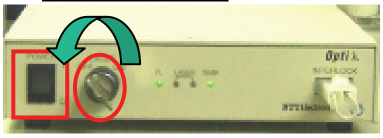
System Shutdown


OLYMPUS FLUOVIEW
File Display Processing Analys
Open
Close
Save
Save As
Property
Logout
Exit
us/Pixel
P:2.0us L:2.116ms
1
Exit
Yes
No

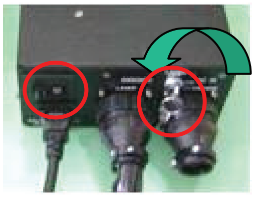
Do you want to exit ?


Defaults
Paint
MSN Explorer
Calculator
All Programs
Help and Support
Search
Run...
2
Log Off
Shut Down
start
Local Disk (F:)
APPENDEX-Kalman - ...

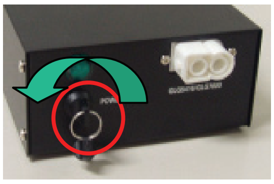
3 **B: LD559nm**

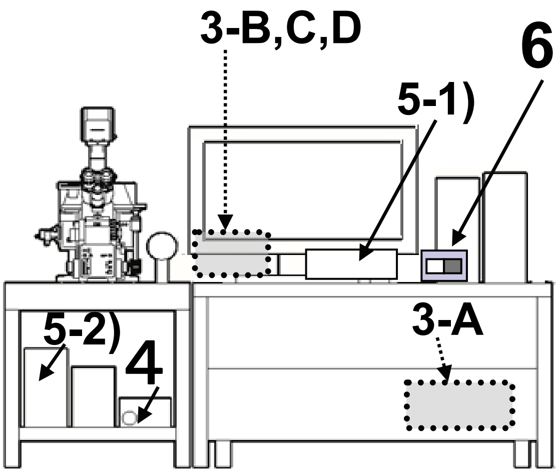


C: MAr laser



D: HeNe (G) laser




3-B,C,D
6
5-1)
5-2)
4
3-A

1. Exit the FV10-ASW software by selecting Exit from the File menu.

2. Shut down Windows.

3. Turn off the laser systems.
   A: MCPSU (405/440/473/635 nm lasers)
   Switch off the system.

   B: LD559nm
   Turn off the key switch to turn off the main power.
   C: Multi Ar laser
   Turn off the key switch to turn off the main power.

   D: HeNe (G) laser (543 nm)
   Turn off the key switch.

4. Switch off the power of the mercury lamp.

5. Set the power switches of the units of microscope to I(ON) according to the following order:
   1)Touch panel controller
   2) IX3-CBH

6. Switch off the central power.

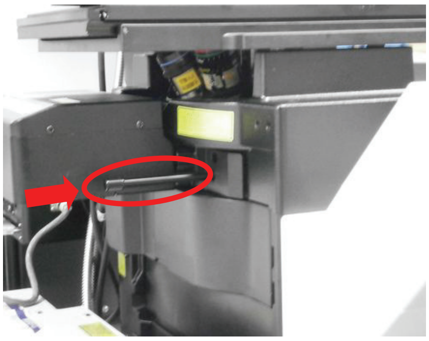
Image Acquisition

— Applications —

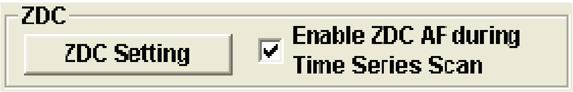
Only for ZDC

XYT Image Acquisition – using ZDC –

※ Just only one shot AF function is described in this manual.



1. Check the checkbox for “Enable ZDC AF during Time Series Scan” in the ZDC group box and then click the ZDC Setting button to open the ZDC Control window.



☑ Enable ZDC AF during Time Series Scan

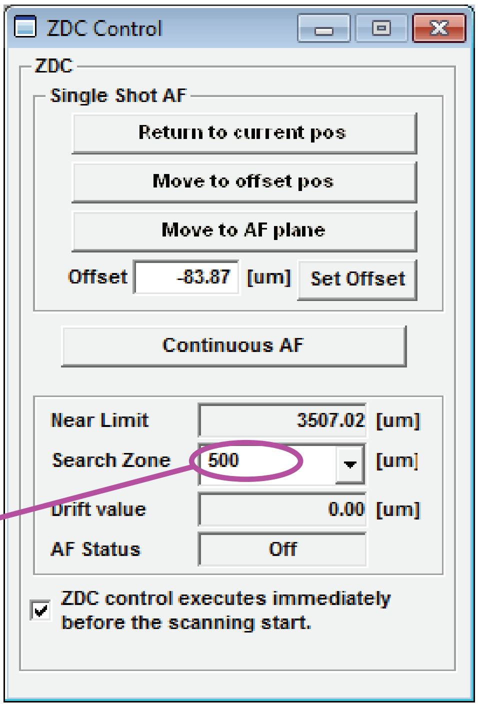
2. Select an objective lens capable of ZDC control and push the Z drift compensation port switching lever to back (IN) position.

   *** Make sure to push the Z drift compensation port switching lever to back position firmly. If the port switching lever is not pushed in firm enough, it may cause malfunction and damage to the ZDC.**

3. Set the image acquisition mode to XYT or XYZT. Adjust the focus on the sample and make image adjustments.

4. Click the ZDC Setting button in the Acquisition Setting window.

5. Click the Set Offset button in the ZDC Control window and set the focus offset value*.



Offset -83.87 [um]

Near Limit 3507.02 [um]

Search Zone 500 [um]

Drift value 0.00 [um]

AF Status Off

☑ ZDC control executes immediately before the scanning start.

Sets an area in which to search for an offset.

*** The “focus offset value” sets the distance between the glass surface, on which the ZDC is detected, and the focus position of the sample.**

6. Start XYT or XYZT image acquisition. ZDC control is carried out at each interval when T series image acquisition is executed.

Notes

ZDC control can be used with non-FreeRUN intervals that are 10 seconds or longer, and the checkbox for “ZDC control executes immediately before the scanning start” in the ZDC group box is checked.

When ZDC control image acquisition is successful, the buzzer issues one beep.
When ZDC control fails, the buzzer issues three beeps.

XYT Image Acquisition

(Photobleaching with a single scanner)

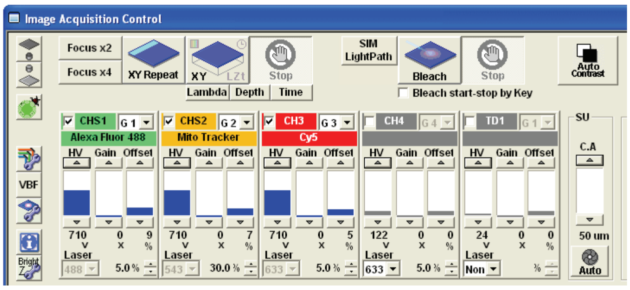
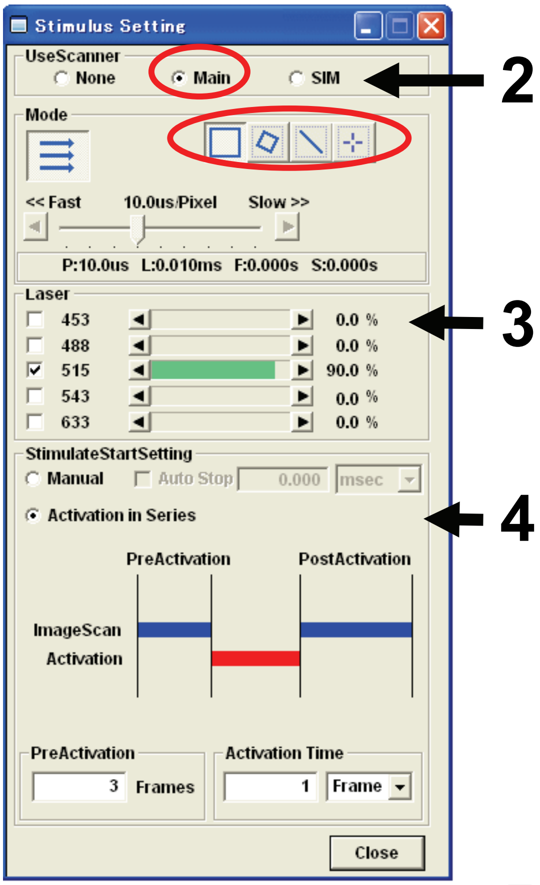


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Bleach start-stop by Key
Auto Contrast
CHS1
Alexa Fluor 488
CHS2
Mito Tracker
CH3
Cy5
CH4
TD1
HV
Gain
Offset
Laser
SU
C.A
50 um
Auto
VBF
Bright

1. Make imaging adjustments.


Stimulus Setting
2
3
4
Close

UseScanner: ○ None ● Main ○ SIM

Mode

<< Fast 10.0us/Pixel Slow >>

P:10.0us L:0.010ms F:0.000s S:0.000s

Laser

☐	453	0.0 %
☐	488	0.0 %
☑	515	90.0 %
☐	543	0.0 %
☐	633	0.0 %

StimulateStartSetting

○ Manual ☐ Auto Stop 0.000 msec

● Activation in Series

PreActivation PostActivation

ImageScan

Activation

PreActivation: 3 Frames

Activation Time: 1 Frame

2. Select Main in the Use Scanner window. Enclose the region to be photobleached using controls in the Mode section.

3. In the Laser section, select the photobleaching power.

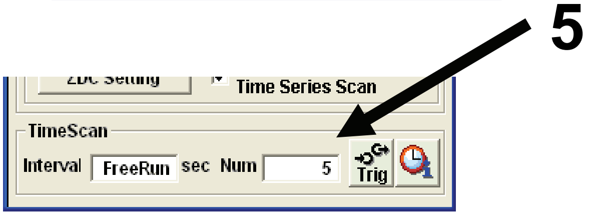
4. Set StimulateStartSetting.

When linking photobleaching with imaging

   4-1. Click the radio button for Activation in Series.

   4-2. Enter the PreActivation and Activation Time fields.

(For manual operation, refer to the following page.)


5
ZDC Setting
Time Series Scan
Trig

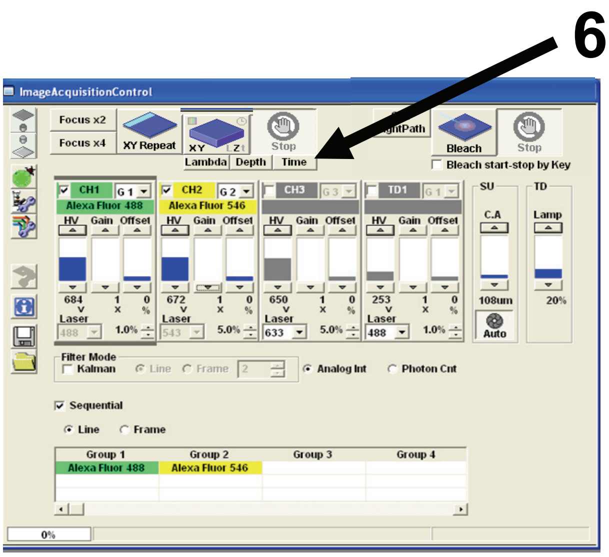
TimeScan

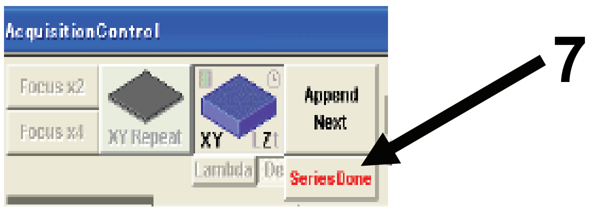
Interval FreeRun sec Num 5

5. Enter the time interval and number.

XYT Image Acquisition

(Photobleaching with a single scanner)


6
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
LightPath
Bleach
Bleach start-stop by Key
CH1
Alexa Fluor 488
CH2
Alexa Fluor 546
CH3
TD1
HV
Gain
Offset
Laser
SU
TD
C.A
Lamp
Auto
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Sequential
Group 1
Group 2
Group 3
Group 4

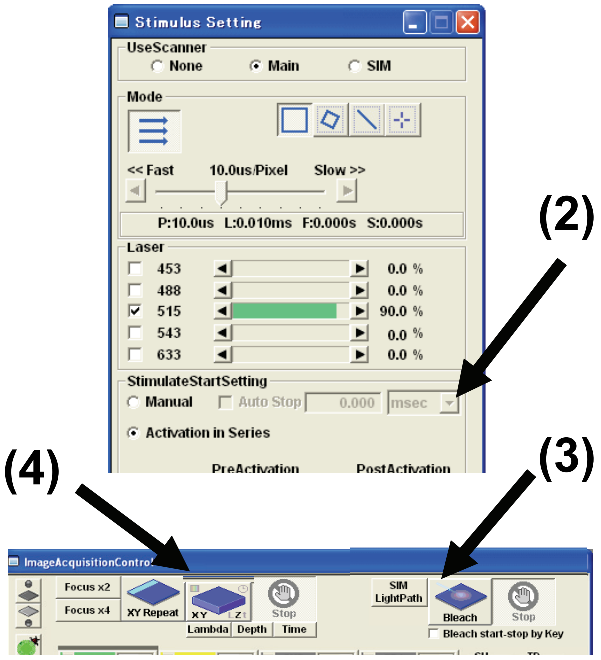

7
AcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Append
Next
SeriesDone

6. With Time selected, click the XYT button to execute image acquisition.

When linking photobleaching with imaging
The region is automatically photobleached at a set time.

7. Clicking SeriesDone causes the window bar of the acquired image to show “2DView-LiveImage(x).”

8. Save the image:
Save the image by selecting Save As from File on the menu bar.
(The Save As types “oib” and “oif” are the dedicated file formats for the FV10-ASW software.)


Stimulus Setting
UseScanner
None
Main
SIM
Mode
<< Fast
10.0us/Pixel
Slow >>
P:10.0us L:0.010ms F:0.000s S:0.000s
Laser
453
488
515
543
633
StimulateStartSetting
Manual
Auto Stop
msec
Activation in Series
PreActivation
PostActivation
(2)
(3)
(4)
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
SIM LightPath
Bleach
Bleach start-stop by Key

When photobleaching manually

(1) Perform operations 1 to 3 on the previous page.

(2) Click the radio button for Manual.
(To set the amount of time the Bleach button is enabled when it is clicked, check the checkbox for Auto Stop and enter the number of seconds.)

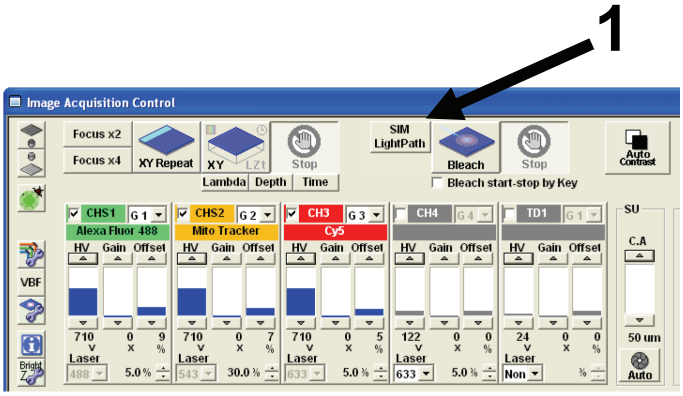
(3) Click the Bleach button.

(4) Observe the image in XY Repeat, XY, or XYT.

Only for SIM

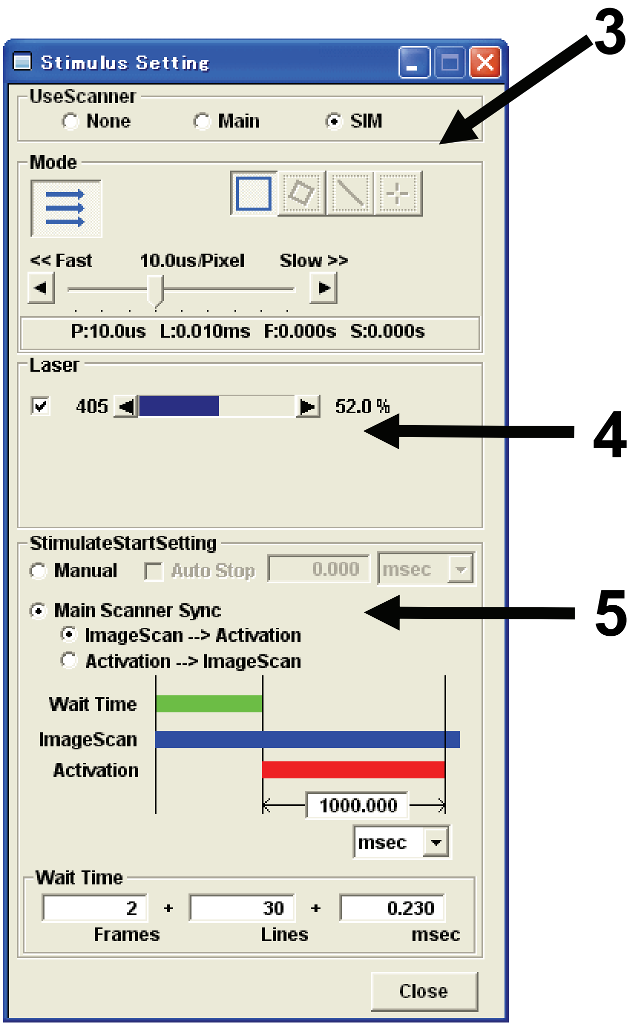
XYT Image Acquisition

(Photobleaching with a SIM scanner)


1
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
CHS1 G1 Alexa Fluor 488
CHS2 G2 Mito Tracker
CH3 G3 Cy5
CH4 G4
TD1 G1
HV Gain Offset
VBF
Bright Z
710 0 9
710 0 7
710 0 5
122 0 0
24 0 0
Laser 488 5.0 %
Laser 543 30.0 %
Laser 633 5.0 %
Laser 633 5.0 %
Laser Non %
SU
C.A
50 um
Auto

1. Click SIM LightPath.

2. Make imaging adjustments.


3
4
5
Stimulus Setting
Mode
Wait Time
ImageScan
Activation

UseScanner ○ None ○ Main ● SIM

<< Fast 10.0us/Pixel Slow >>

P:10.0us L:0.010ms F:0.000s S:0.000s

Laser ☑ 405 52.0 %

StimulateStartSetting

○ Manual ☐ Auto Stop 0.000 msec

● Main Scanner Sync

● ImageScan --> Activation

○ Activation --> ImageScan

1000.000 msec

Wait Time 2 Frames + 30 Lines + 0.230 msec

Close

3. Select SIM in the Use Scanner window. Enclose the region to be photobleached using controls in the Mode section.

4. In the Laser section, select the photobleaching power.

5. Set StimulateStartSetting.

When photobleaching manually

5-1. Click the radio button for Manual.

(To set the amount of time the Bleach button is enabled when it is clicked, check the checkbox for Auto Stop and enter the number of seconds.)

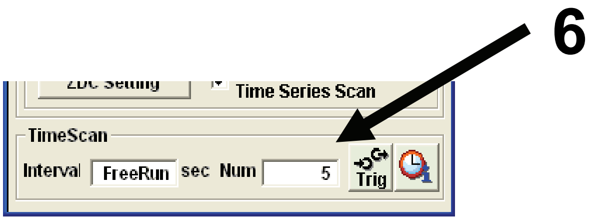
When linking photobleaching with imaging

5-1. Click the radio button for Main Scanner Sync and for ImageScan → Activation.

5-2. Enter the values for Activation and Wait Time.

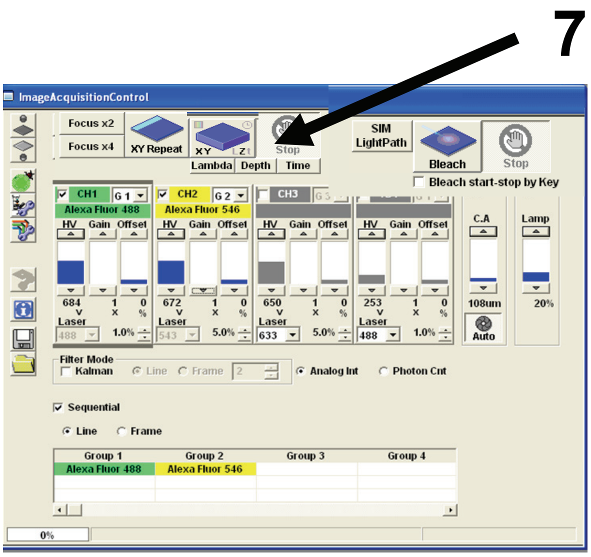
XYT Image Acquisition

(Photobleaching with a SIM scanner)


6
Time Series Scan
TimeScan
Trig

Interval FreeRun sec Num 5

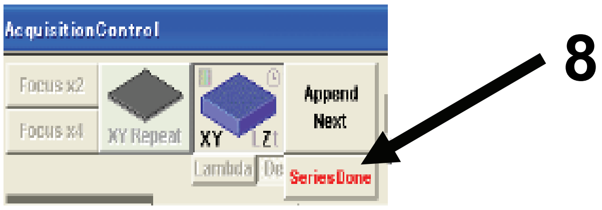
6. Enter the time interval and number.


7
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
CH1
Alexa Fluor 488
CH2
Alexa Fluor 546
CH3
HV
Gain
Offset
Laser
C.A
Lamp
108um
20%
Auto
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Sequential
Line
Frame
Group 1
Group 2
Group 3
Group 4
0%

7. With Time selected, click the XYT button to execute image acquisition.

When photobleaching manually
Click the Bleach button when you want to start photobleaching.

When linking photobleaching with imaging
The region is automatically photobleached at the set time.


8
AcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Zt
Lambda
Append
Next
SeriesDone

8. Clicking SeriesDone causes the window bar of the acquired image to show “2DView-LiveImage(x).”

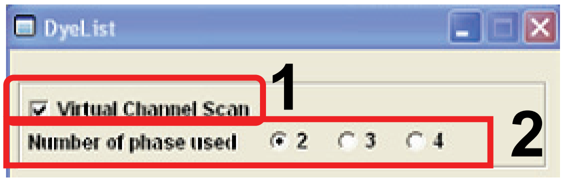
9. Save the image:
Save the image by selecting Save As from File on the menu bar.
(The Save As types “oib” and “oif” are the dedicated file formats for the FV10-ASW software.)

XY Image Acquisition

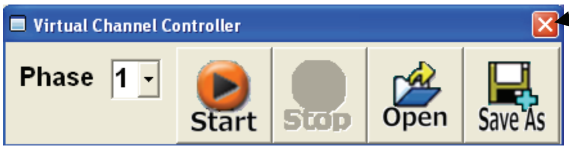
(Four-color staining with virtual channel)

Acquiring a four-color staining image with virtual channel scan

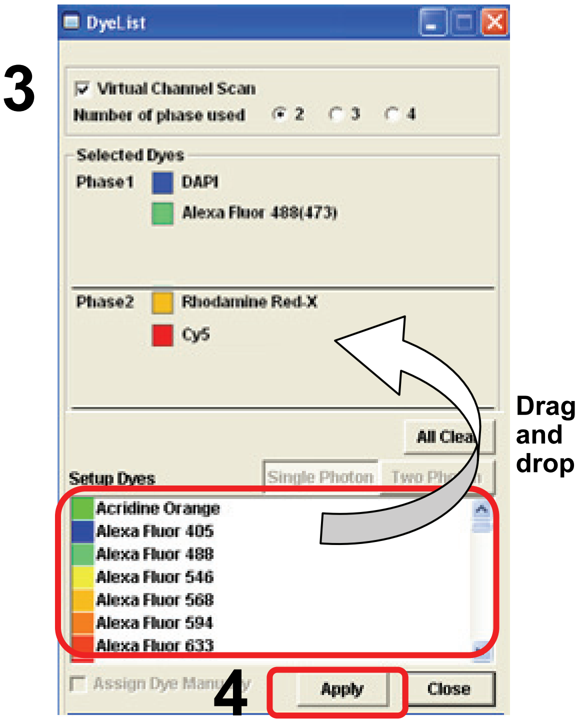
Example: DAPI and Alexa488 for phase 1 / RodaminRed and Cy5 for phase 2



☑ Virtual Channel Scan
Number of phase used ● 2 ○ 3 ○ 4


Virtual Channel Controller
Phase
1
Start
Stop
Open
Save As

1. Check the checkbox for ☑ Virtual Channel Scan in DyeList to automatically open the Virtual Channel Controller.
2. In “Number of phase used,” select “2” as the phase count.


3
DyeList
All Clear
Single Photon
Two Photon
Drag and drop
4
Apply
Close

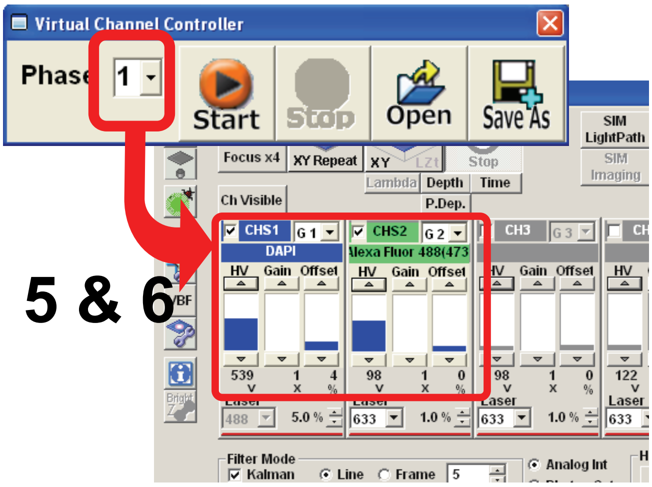
☑ Virtual Channel Scan
Number of phase used ● 2 ○ 3 ○ 4

Selected Dyes
Phase1 🟦 DAPI
🟩 Alexa Fluor 488(473)
Phase2 🟧 Rhodamine Red-X
🟥 Cy5

Setup Dyes
🟩 Acridine Orange
🟦 Alexa Fluor 405
🟩 Alexa Fluor 488
🟨 Alexa Fluor 546
🟧 Alexa Fluor 568
🟧 Alexa Fluor 594
🟥 Alexa Fluor 633

☐ Assign Dye Manually

3. Drag a dye from the dye list to each Phase.
4. To set the fluorescent dyes and the phase count, click Apply.


5 & 6
Virtual Channel Controller
Phase
1
Start
Stop
Open
Save As
SIM LightPath
SIM Imaging
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
P.Dep.
Ch Visible
CHS1
G1
DAPI
CHS2
G2
Alexa Fluor 488(473)
CH3
G3
HV
Gain
Offset
539
1
4
98
1
0
98
1
0
122
Laser
488
5.0 %
633
1.0 %
633
1.0 %
633
Filter Mode
Kalman
Line
Frame
5
Analog Int

5. Select Phase1 to display DAPI and Alexa488 in Image Acquisition Control.

   * The slit width, filter, and DM settings are switched to DAPI and Alexa488.

   Adjust the image.
6. Select Phase2 and adjust the image again.
7. To acquire the image, click Virtual Channel Controller Start .
8. Save the image.

The use of this function does not reflect the LUT setting.

After image acquisition finishes, change the LUT setting by using LUT Setting .

HDRi Image Acquisition

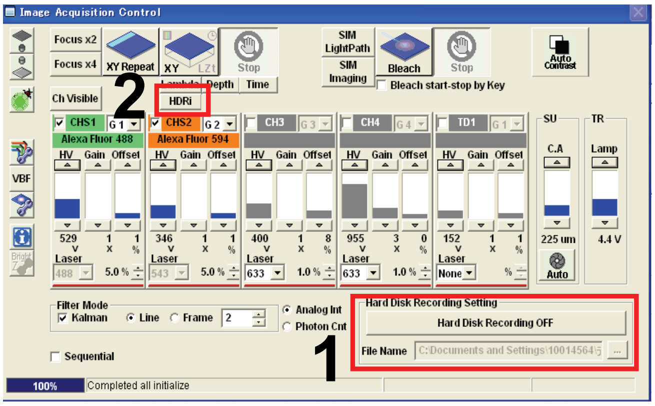
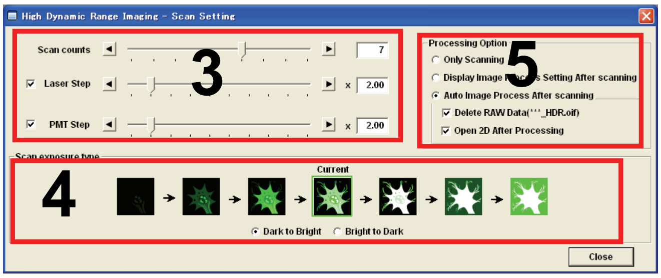


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
SIM LightPath
SIM Imaging
Bleach
Stop
Auto Contrast
Bleach start-stop by Key
Ch Visible
2
HDRi
Depth
Time
CHS1
CHS2
CH3
CH4
TD1
Alexa Fluor 488
Alexa Fluor 594
HV
Gain
Offset
SU
TR
C.A
Lamp
VBF
225 um
4.4 V
Auto
Laser
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Sequential
Hard Disk Recording Setting
Hard Disk Recording OFF
File Name
C:\Documents and Settings\100145645
1
100%
Completed all initialize


High Dynamic Range Imaging - Scan Setting
Scan counts
Laser Step
PMT Step
7
x 2.00
x 2.00
3
Processing Option
Only Scanning
Display Image Process Setting After scanning
Auto Image Process After scanning
Delete RAW Data(***_HDR.oif)
Open 2D After Processing
5
Scan exposure type
Current
4
Dark to Bright
Bright to Dark
Close

HDRI Scan Setting window

1. After finishing image adjustment, set the save destination for hard disk recording.

2. Turn on **HDRi** in the Image Acquisition Control to open the HDRI Scan Setting window.

3. Set the condition for HDRi.
   - Scan count: number of scans (2 to 10)
   - Laser Step: increase/decrease rate of laser output
   - PMT Step: increase/decrease rate of HV

   *** The value of laser output in Acquisition Setting and the value of HV in Image Acquisition Control are applied to the basic values for HDRi.**

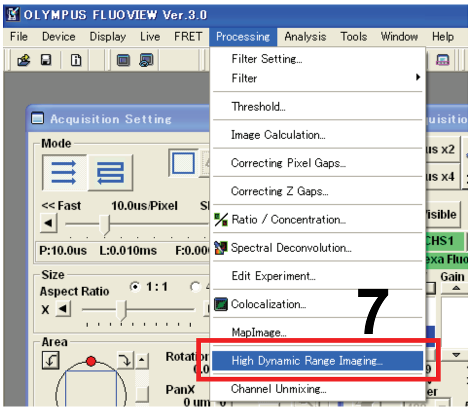
4. Select “Dark to Bright” or “Bright to Dark” and click the icon of acquisition sequence which is given the basic values for HDRi. “Current” is shown on the top of the selected icon.

Example of settings

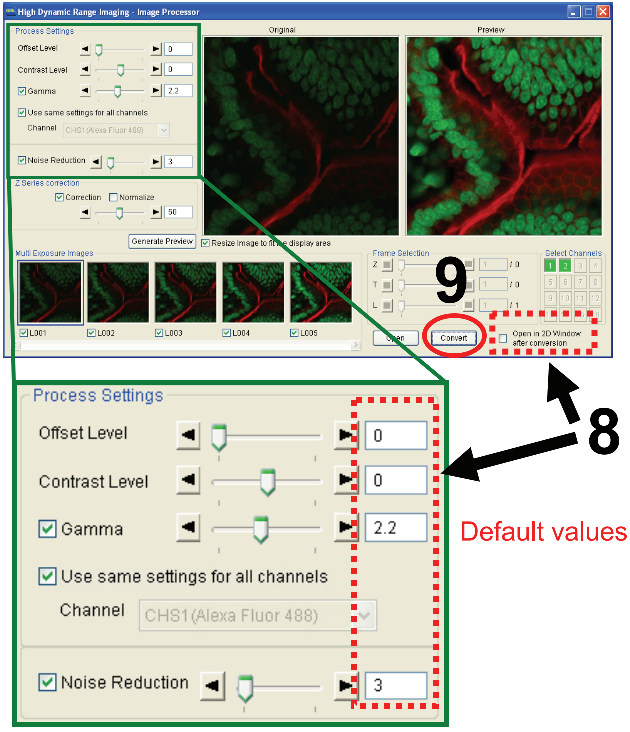
<Basic values>
Laser output in Acquisition Setting: 10.0 (%)
HV in Image Acquisition Control: 500 (V)
<Condition settings for HDRi >
Scan count = 5, Laser Step = x1.2, PMT Step = x1.2,
Dark to Bright, “Current” icon = 3rd from the left
<Setting results>
Laser output (%) : 6.9 -> 8.3 -> 10.0 -> 12.0 -> 14.4
HV (V) : 347 -> 417 -> 500 -> 600 -> 720

5. In the Processing Option section, set the process to be executed after image acquisition finishes.
   - **Only Scanning**: Only executes scanning.
   - **Display Image Process Setting After scanning**: Displays the HDRi Image Processor window after scanning finishes.
   - **Auto Image Process After scanning**: Executes HDRi image processing automatically after scanning finishes. (The parameters used when HDRi Image Processor was last used are applied.)

HDRi Image Acquisition


OLYMPUS FLUOVIEW Ver.3.0
File Device Display Live FRET Processing Analysis Tools Window Help
Filter Setting...
Filter
Threshold...
Image Calculation...
Correcting Pixel Gaps...
Correcting Z Gaps...
Ratio / Concentration...
Spectral Deconvolution...
Edit Experiment...
Colocalization...
MapImage...
High Dynamic Range Imaging...
Channel Unmixing...
Acquisition Setting
Mode
7

HDRi Image Processor window


High Dynamic Range Imaging - Image Processor
Original
Preview
Z Series correction
Generate Preview
Multi Exposure Images
L001
L002
L003
L004
L005
Frame Selection
Select Channels
Open
Convert
Open in 2D Window after conversion
9
8
Default values

Process Settings
Offset Level: 0
Contrast Level: 0
☑ Gamma: 2.2
☑ Use same settings for all channels
Channel: CHS1(Alexa Fluor 488)
☑ Noise Reduction: 3

Offset Level: The intensity less then Offset value is regarded as 0. The low intensity noise on the image can be eliminated.
Contrast Level: The grayscale of entire images can be adjusted.
Gamma: Gamma correction.
Use same settings for all channels: If it is ticked, the same settings are performed in the image processing of all channels.
Noise Reduction: The noise can be reduced without losing the characteristics of the sample.

6. To start scanning images, click the XY button. Scanned images are saved in the set save destination for hard disk recording.

   (File names are assigned with the format ***_**HDR.oif**.)

When Auto Image Process After scanning is selected in step 6, images are saved after being automatically formatted in HDRI (Files named ***_.oif).

7. From the menu, select Processing and then High Dynamic Range Image to open the HDRi Image Processor window.

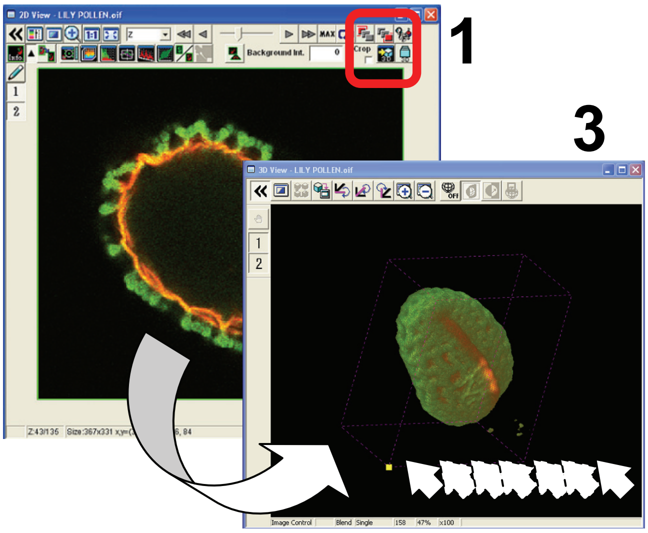
When Display Image Process Setting After scanning is selected in step 6, the window automatically opens.

8. Set the Offset Level, Contrast Level, Gamma and Noise Reduction, and then check the checkbox of “Open in 2D Window after conversion”.

9. To create HDRi images, click the Convert button.

— Supplemental Operations —

3D Image Analysis 3D image creation/cross-sectional observation

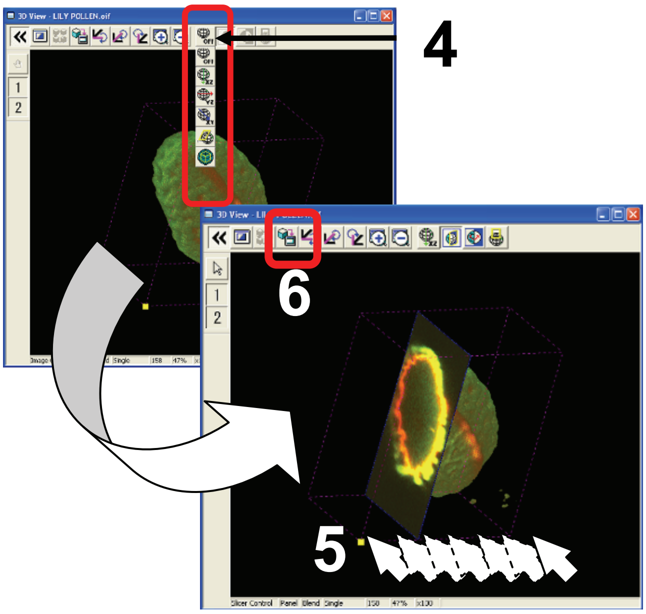

1
3
2D View - LILY POLLEN.oif
3D View - LILY POLLEN.oif

Observe an image from a desired angle.

1. Click 3D of the 2D View-(file name) image.

2. A 3D View is created.

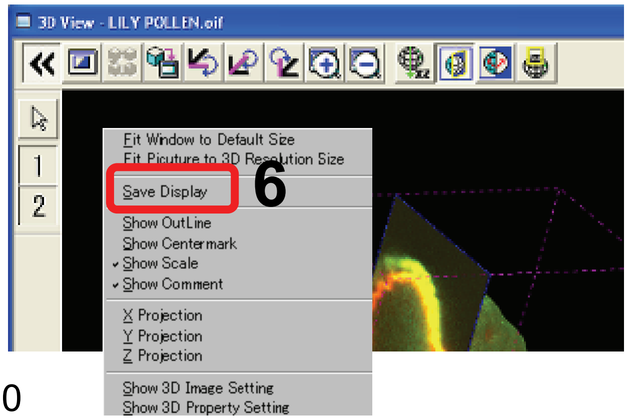
3. To observe the object from a desired angle, move the 3D object by dragging the mouse on the image.


4
6
5
3D View - LILY POLLEN.oif

Observe the desired cross-section of an image.

4. Click OFF and then select XZ.

5. To observe the desired vertical cross-section, move the object right and left by dragging the mouse on the image.


3D View - LILY POLLEN.oif
Fit Window to Default Size
Fit Picuture to 3D Resolution Size
Save Display
6
Show OutLine
Show Centermark
Show Scale
Show Comment
X Projection
Y Projection
Z Projection
Show 3D Image Setting
Show 3D Property Setting

Save the 3D image.

6. Click to create a 2D image. Save the image by right-clicking.

(Or, right click on the 3D image in order to select Save Display to save the view, including the ROI being displayed, in BMP format.)

3D Image Analysis Detailed settings for 3D image creation

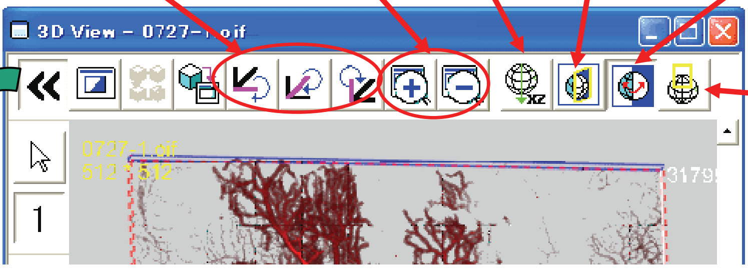
Cut View button
Projects a cross-sectional view on a slice when the slice graphics mode is set.

Image zoom in/out

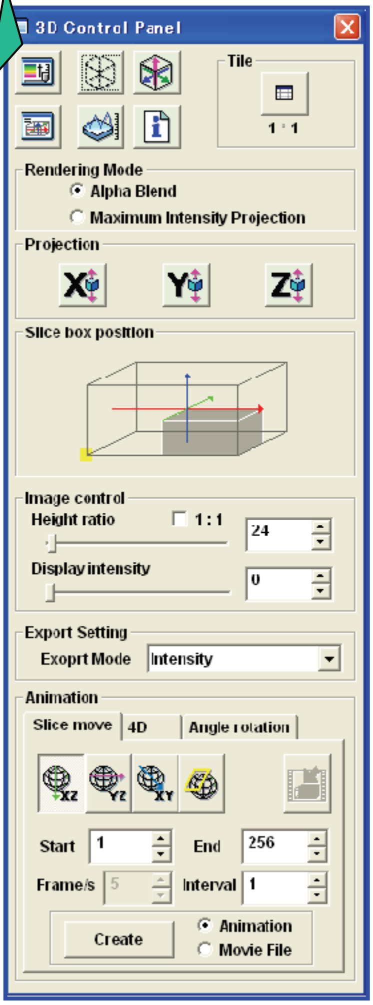
3D image rotation

Slice button

Change View Region button
Switches between the sliced 3D image view region and the hidden region.


3D View – 0727-1.oif
XZ
0727-1.oif
512 * 512
1
3179

Orthogonal View button
Allows for observation with the view point fixed at the front of a sliced cross-section.


3D Control Panel
Tile
1 : 1
X
Y
Z
Slice move
4D
Angle rotation
XZ
YZ
XY
Create

Rendering Mode
● Alpha Blend
○ Maximum Intensity Projection

Projection

Slice box position

Image control
Height ratio ☐ 1:1 24
Display intensity 0

Export Setting
Exoprt Mode Intensity

Animation
Start 1 End 256
Frame/s 5 Interval 1
● Animation
○ Movie File

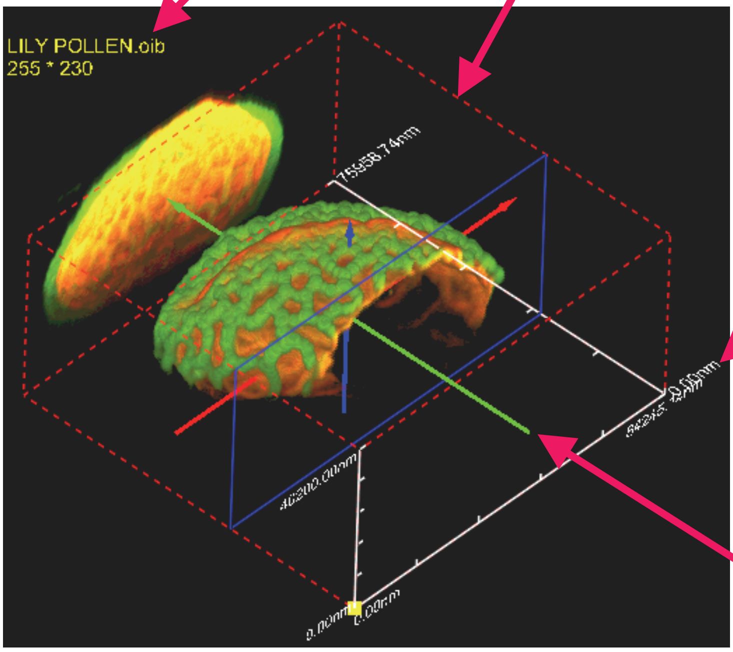
3D Image Settings (LUT adjustment, image adjustment, etc.)

3D Property Settings (image graphics setting such as scale bars and comments)

Graphics ON/OFF

Comment

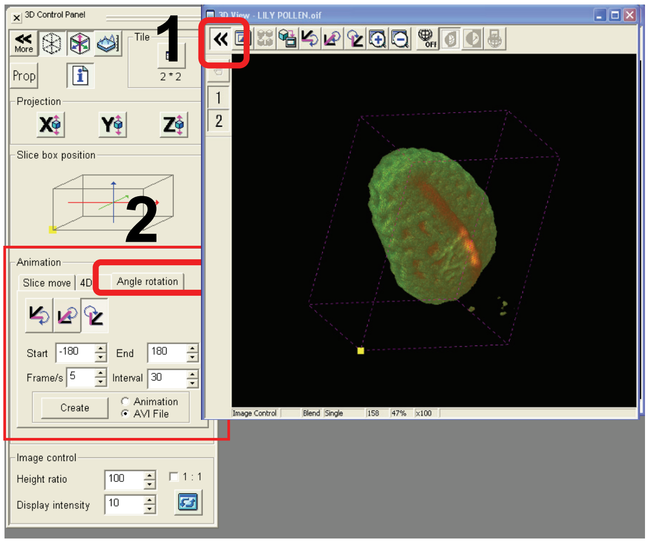
Outline

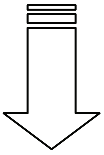

LILY POLLEN.oib
255 * 230
75958.74nm
40200.00nm
0.00nm
0.00nm
0.00nm

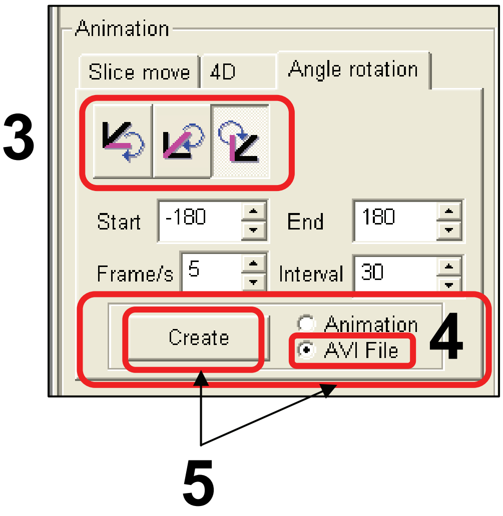
Scale

Center mark

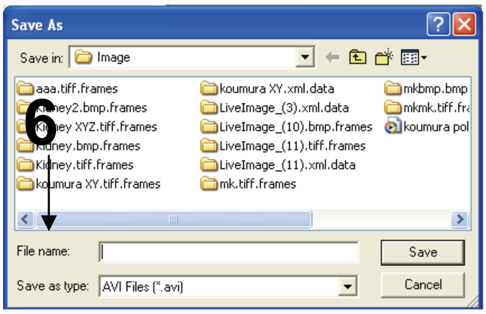
3D Image Analysis How to create a file


1
2
3D Control Panel
More
Tile
2*2
Prop
Projection
Slice box position
Animation
Slice move
4D
Angle rotation
Start -180
End 180
Frame/s 5
Interval 30
Create
Animation
AVI File
Image control
Height ratio 100
1 : 1
Display intensity 10
3D View - LILY POLLEN.oif




3
4
5
Animation
Slice move
4D
Angle rotation
Create

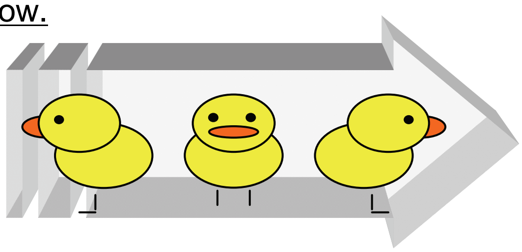
Start: -180
End: 180
Frame/s: 5
Interval: 30
○ Animation
● AVI File


6
Save As
Save in: Image
aaa.tiff.frames
Kidney2.bmp.frames
Kidney XYZ.tiff.frames
Kidney.bmp.frames
Kidney.tiff.frames
koumura XY.tiff.frames
koumura XY.xml.data
LiveImage_(3).xml.data
LiveImage_(10).bmp.frames
LiveImage_(11).tiff.frames
LiveImage_(11).xml.data
mk.tiff.frames
mkbmp.bmp
mkmk.tiff.fra
koumura pol
Save
Cancel

File name:
Save as type: AVI Files (*.avi)

To save a rotation file as a movie, render the image in 3D with the following procedure.

As an example, an image is rotated 180º below.



1. Click the « button.

2. Click the Angle rotation tab.

3. Select the rotation axis.

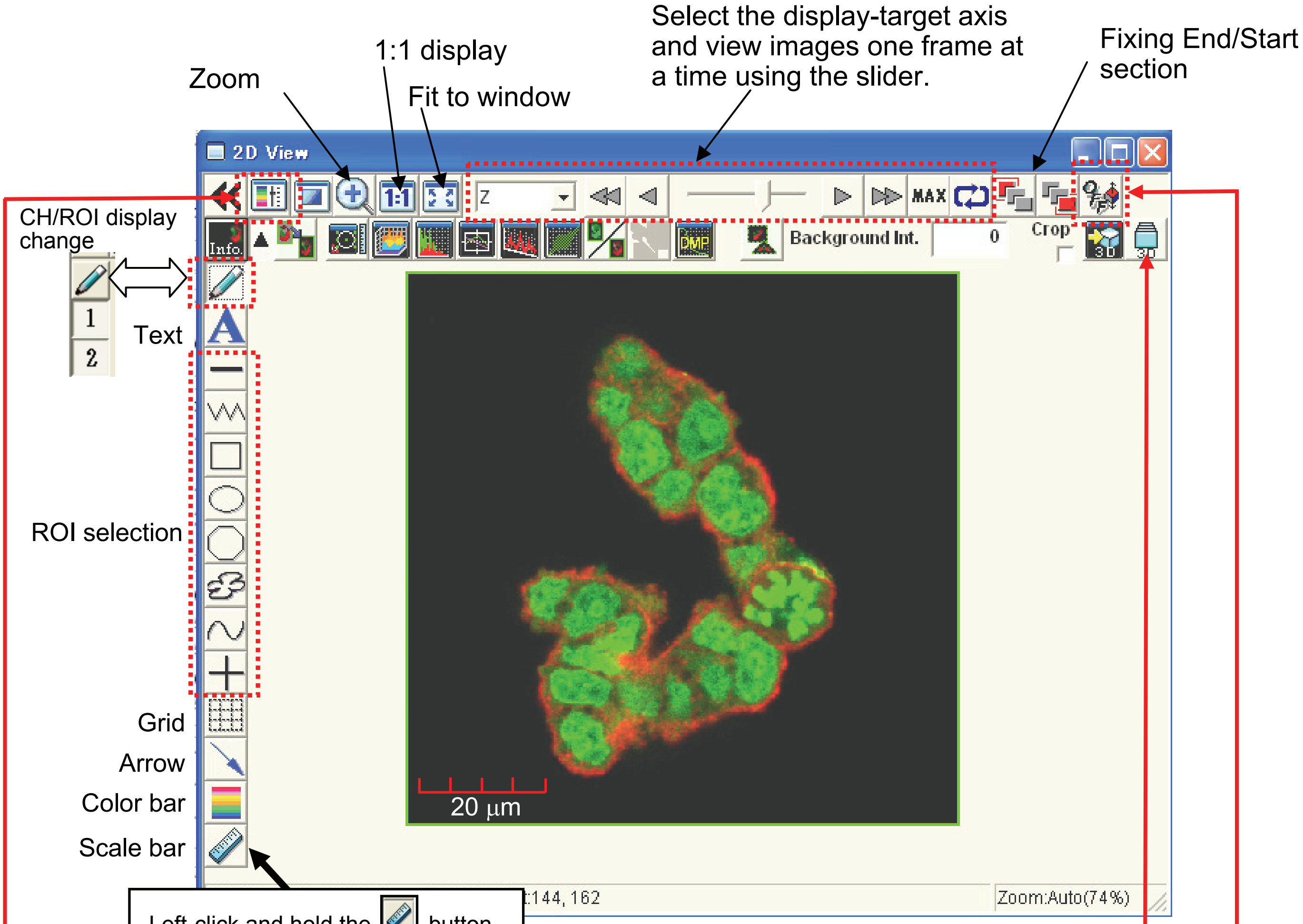
4. Enter the rotation angle.

Start: starting angle / End: ending angle
Frame/s: rotation speed / Interval: angle interval

5. Select AVI File and then click Create.

6. Enter a file name and then click Save.

2D View Window


Zoom
1:1 display
Fit to window
Fixing End/Start section
CH/ROI display change
1
2
Text
ROI selection
Grid
Arrow
Color bar
Scale bar
2D View
Z
MAX
Info.
Background Int.
0
Crop
3D
20 µm
:144, 162
Zoom:Auto(74%)

Select the display-target axis and view images one frame at a time using the slider.

Left-click and hold the button to drag it to a place on the image and drop it there.
(A straight line can be drawn by pressing Shift when left-clicking.)

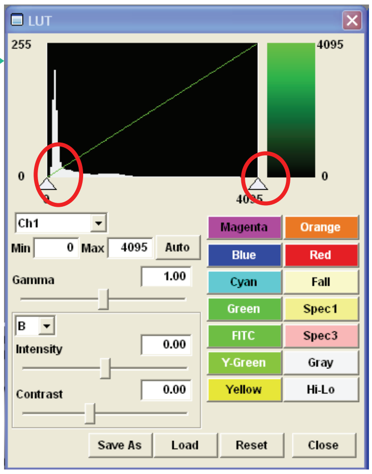
Creation of rotating 3D animation

Creates a rotating 3D animation based on the X axis (standard animation).

Creates a rotating 3D animation based on the Y axis (standard animation)

Displays the 3D Animation window that can be used to customize the settings of the rotating 3D animation creation process.
Enter settings and click New Image to create a 3D animation.

LUT setting (edits image brightness)


LUT
255
4095
0
0
4095
Ch1
Min
0
Max
4095
Auto
Gamma
1.00
B
Intensity
0.00
Contrast
0.00
Magenta
Orange
Blue
Red
Cyan
Fall
Green
Spec1
FITC
Spec3
Y-Green
Gray
Yellow
Hi-Lo
Save As
Load
Reset
Close

Contrast can be freely set by moving the cursor right or left, or by entering values in Min and Max.

Projection mode change

Projection Off button: No projection displayed.

Z Intensity Projection button: Displays Z Intensity Projection images.

T Intensity Projection button: Displays T Intensity Projection images.

λ Intensity Projection button: Displays λ Intensity Projection images.

Z Topographic Projection button: Displays Z Topographic Projection images.

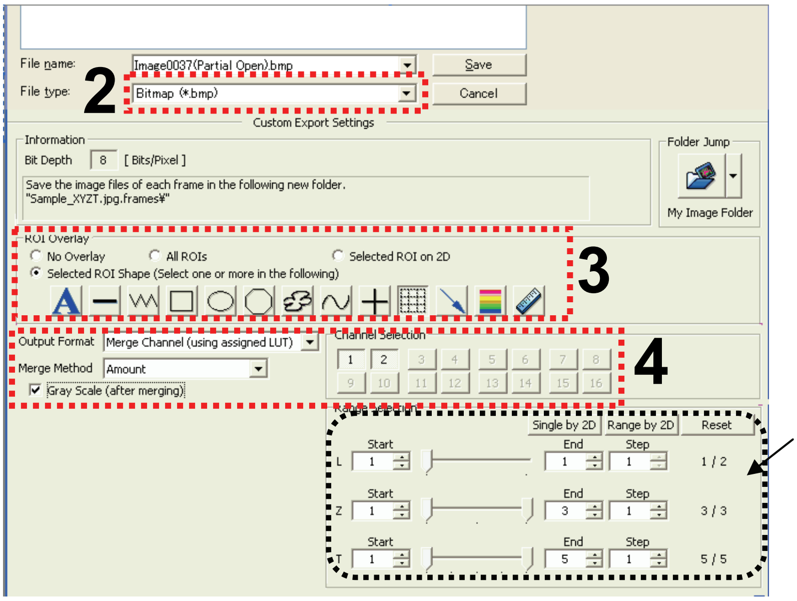
λ Topographic Projection button: Displays λ Topographic Projection images.

T Series Average button: Displays T series average images.

Acquired Image Export

Saving images as Bitmap (*.bmp), JPEG (*.jpg, *.jpeg), TIFF (*.tif, *.tiff), or PNG (*.png)

1. Open the Live View window or 2D View window of the image to be saved. Right-click on the image and then select Export.
2. Select a file format to use for the export.
3. When saving a ROI in an image, such as a scale bar, select All ROIs or Selected ROI Shape.
4. Select the output mode of the image. In Channel Selection, select an output channel.
    - Merging images
    - LUT images for each CH
    - Raw data (black and white) images
5. To save the file, click Save.


2
3
4
Save
Cancel
Custom Export Settings
Information
Folder Jump
My Image Folder
ROI Overlay
○ No Overlay
○ All ROIs
○ Selected ROI on 2D
Channel Selection
Range Selection
Single by 2D
Range by 2D
Reset
Start
End
Step
L
Z
T

File name: Image0037(Partial Open).bmp

File type: Bitmap (*.bmp)

Bit Depth 8 [Bits/Pixel]

Save the image files of each frame in the following new folder.
"Sample_XYZT.jpg.frames¥"

● Selected ROI Shape (Select one or more in the following)

Output Format: Merge Channel (using assigned LUT)

Merge Method: Amount

☑ Gray Scale (after merging)

L: Start 1, End 1, Step 1, 1 / 2

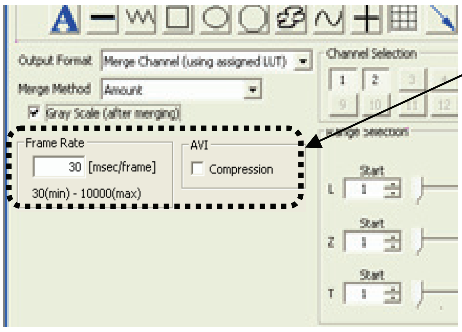
Z: Start 1, End 3, Step 1, 3 / 3

T: Start 1, End 5, Step 1, 5 / 5

Range selection
Specify the Start and End values of axes to be read (L/Z/T).

Saving images in movie format (AVI or MOV) (The AVI or MOV format is available for XYZ, XYT, XYλ, XZT, XλT, and XλZ images.)

1. Open the Live View window or 2D View window of the image to be saved. Right-click on the image and then select Export.
2. Select a movie format and enter the frame rate in the Frame Rate field.
3. To save the file, click Save.


Channel Selection
Range Selection
Start
L
Z
T

Output Format: Merge Channel (using assigned LUT)

Merge Method: Amount

☑ Gray Scale (after merging)

Frame Rate: 30 [msec/frame] 30(min) - 10000(max)

AVI: ☐ Compression

Frame Rate
The frame rate of the movie and whether to set compression or not can be set.
(AVI: Sets whether to compress the AVI file.)

Only for SPD

Adjustment of Acquisition Wavelength Range (VBF)

The wavelength range of waves acquired in Ch1 and Ch2 can be adjusted by using the **Variable Barrier Filter**.

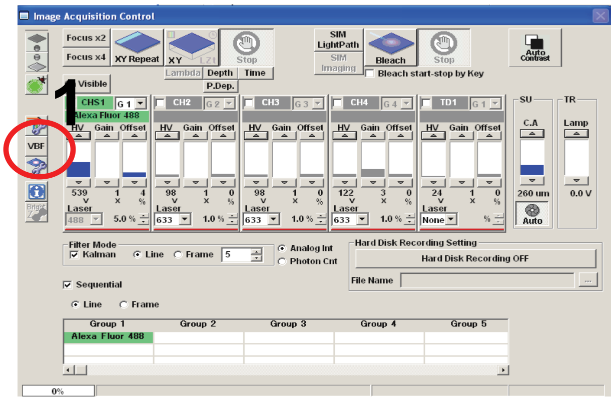
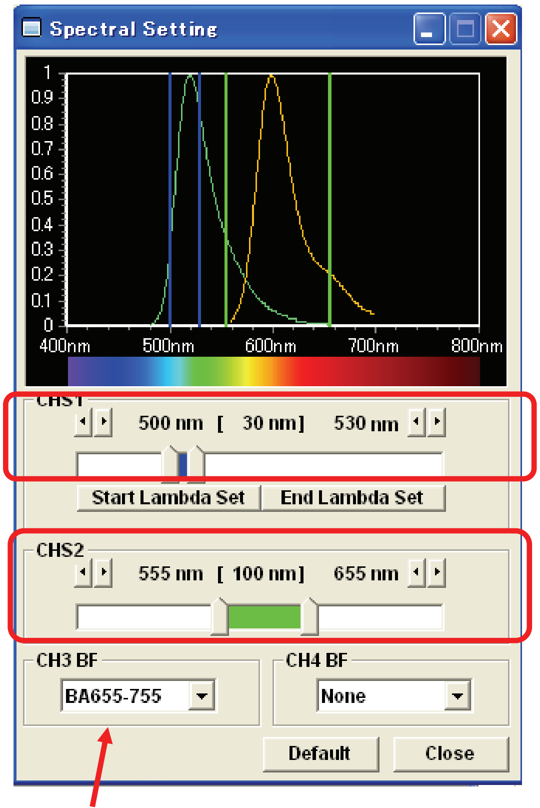


Image Acquisition Control
1
VBF
CHS1
Alexa Fluor 488
Group 1
Group 2
Group 3
Group 4
Group 5

1. Click the VBF button to open the Spectral Setting window.
2. Using or 500um, move the position to adjust the wavelength of the wave to be acquired.

Spectral Setting window


Spectral Setting
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400nm
500nm
600nm
700nm
800nm
Start Lambda Set
End Lambda Set
Default
Close

CHS1 500 nm [30 nm] 530 nm

CHS2 555 nm [100 nm] 655 nm

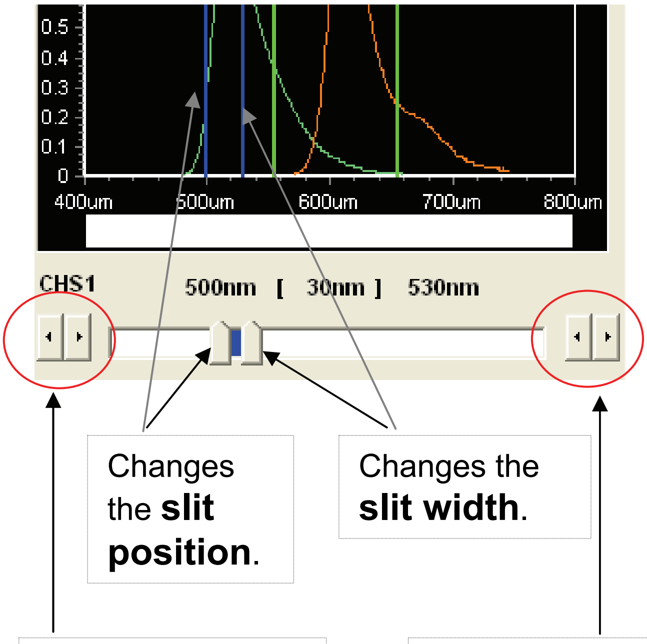
CH3 BF BA655-755

CH4 BF None

Slit adjustment for Ch1

Slit adjustment for Ch2

Fluorescent filter selection for Ch3


0.5
0.4
0.3
0.2
0.1
0
400um
500um
600um
700um
800um
CHS1
500nm [30nm] 530nm

Changes the **slit position**.

Changes the **slit width**.

Changes the **slit position** in 1 nm increments.

Changes the **slit width** in 1 nm increments.

Hard Disk Recording

- This function automatically saves acquired images in a preset save destination.
- When series imaging is executed, you can check images up to the immediately preceding one through the Viewer window in the middle of the image acquisition process.

[Hard disk recording conditions]

Maximum number of image acquisition	32,766 times (number of images in series)
Save destination	Only local drives are guaranteed (D drive)
Other conditions	XYT images below 128×128 in size. Intervals of 1 second or more are required. Disabled for the reciprocating scan mode. **The review function is only available for interval operation.**

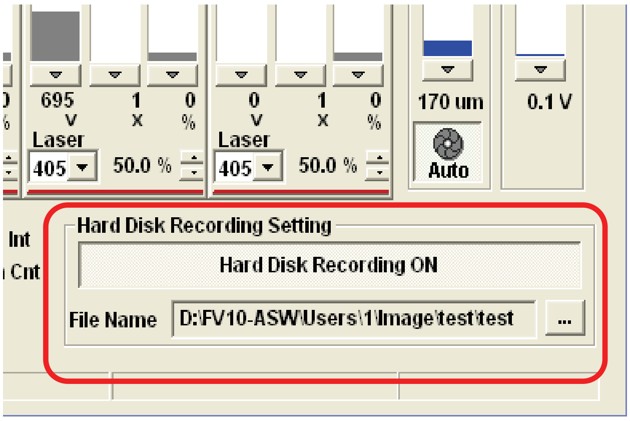
How to acquire images

1. Set the image adjustment and condition settings.

2. Click the Hard Disk Recording OFF button. The button appears as Hard Disk Recording On and the Viewer window opens.

3. Click the ... button next to the File Name textbox. Set the save destination and file name and then click Open.

4. To acquire images, click the XY LZt button.



Hard Disk Recording Setting

Hard Disk Recording ON

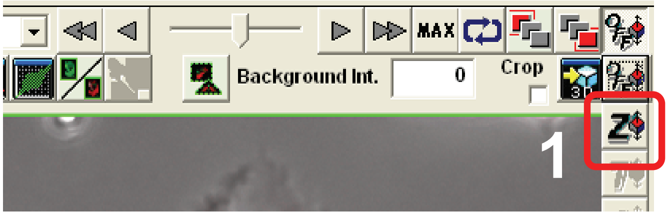
File Name D:\FV10-ASW\Users\1\Image\test\test ...

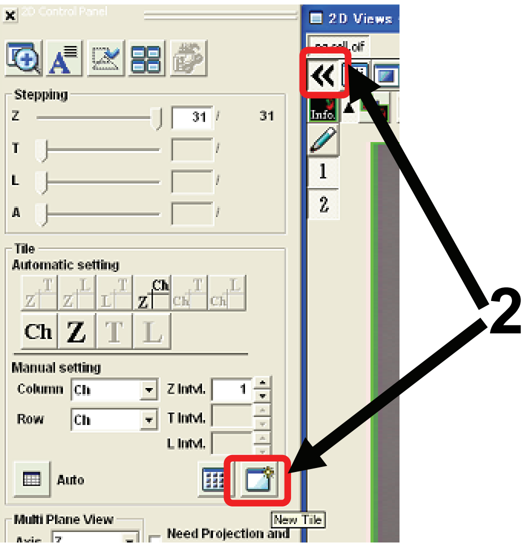
Viewing images in the middle of the image acquisition process

1. After image acquisition starts, bring the Viewer window to the front.
2. Double-click an image file during the acquisition process with the view set to Thumbnail in the Explorer window.

(When image files are not displayed in the Thumbnail view during the acquisition process, right-click and select Refresh.)

A. Creation of 2D Z Projection Images
B. Reloading Set Conditions


MAX
Background Int.
0
Crop
1

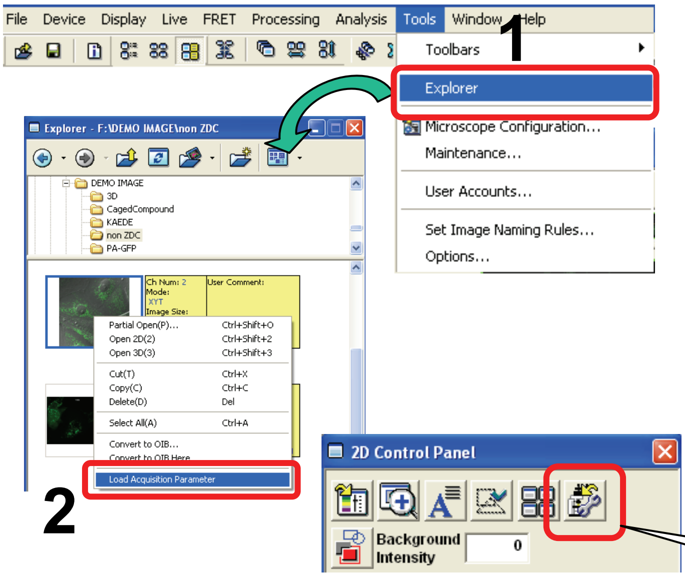

2D Control Panel
2D Views
Info.
Stepping
Z
31
31
T
L
A
Tile
Automatic setting
Ch
Z
T
L
Manual setting
Column
Ch
Z Intvl.
1
Row
Ch
T Intvl.
L Intvl.
Auto
New Tile
Multi Plane View
Need Projection and
2

A. Creation of 2D Z projection images

1. Open an original image and then select Z from the Projection button in the 2D View to open the Z projection image.

2. Click the New Tile button in the 2D Control Panel (opened by clicking «) to create a 2D image of the Z projection image.

3. Save the image.


File
Device
Display
Live
FRET
Processing
Analysis
Tools
Window
Help
Toolbars
Explorer
Microscope Configuration…
Maintenance…
User Accounts…
Set Image Naming Rules…
Options…
1
Explorer - F:\DEMO IMAGE\non ZDC
DEMO IMAGE
3D
CagedCompound
KAEDE
non ZDC
PA-GFP
Ch Num: 2
Mode:
XYT
Image Size:
User Comment:
Partial Open(P)…
Ctrl+Shift+O
Open 2D(2)
Ctrl+Shift+2
Open 3D(3)
Ctrl+Shift+3
Cut(T)
Ctrl+X
Copy(C)
Ctrl+C
Delete(D)
Del
Select All(A)
Ctrl+A
Convert to OIB…
Convert to OIB Here
Load Acquisition Parameter
2
2D Control Panel
Background Intensity
0

B. Reloading set conditions

Loads image acquisition conditions from past image data in order to duplicate them.

1. Select Explorer from Tools.

2. Right-click on a thumbnail image from which to reload set conditions, and then select Load Acquisition Parameter.

3. The conditions are reloaded.

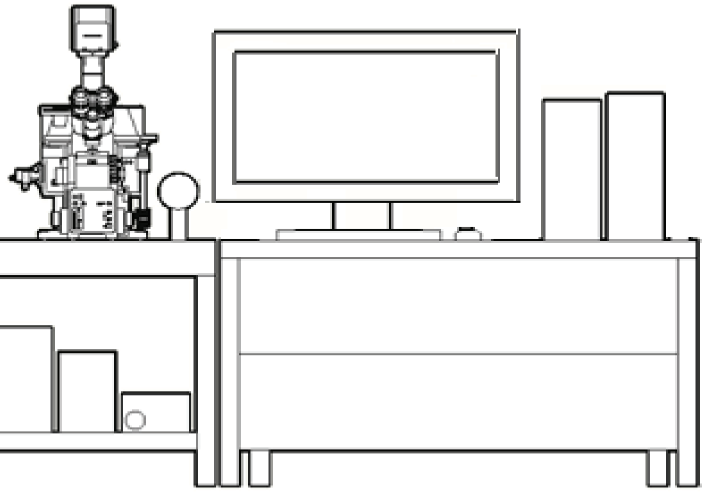
Conditions can be reloaded by clicking the Load Scan Data button while the image from which to reload conditions is open.

Laser Scanning Biological Microscope

FV1200

IX81

Quick Start



FV1200 IX81
Quick Start
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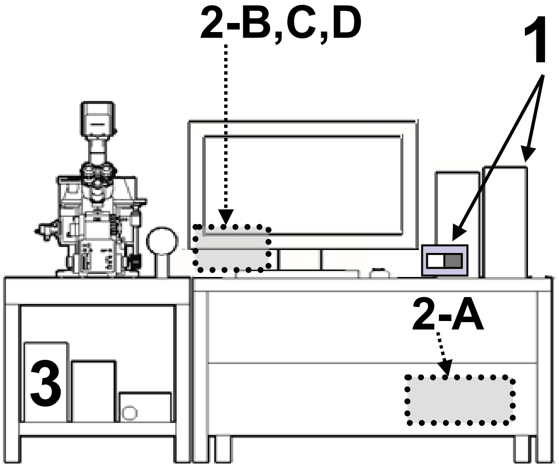
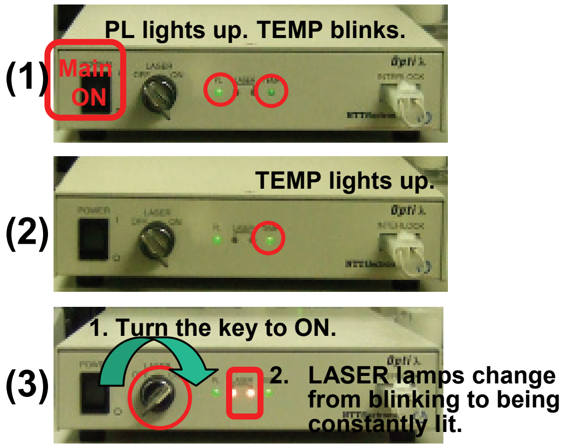
Image Acquisition

— Fundamentals —

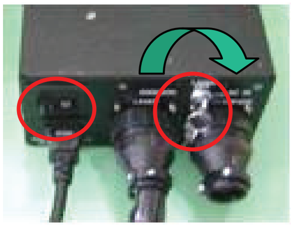
System Startup



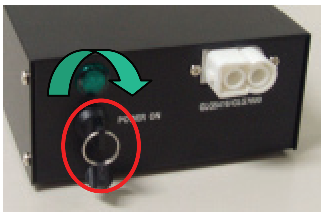
B: LD559 laser


(1)
PL lights up. TEMP blinks.
Main ON
(2)
TEMP lights up.
(3)
1. Turn the key to ON.
2. LASER lamps change from blinking to being constantly lit.

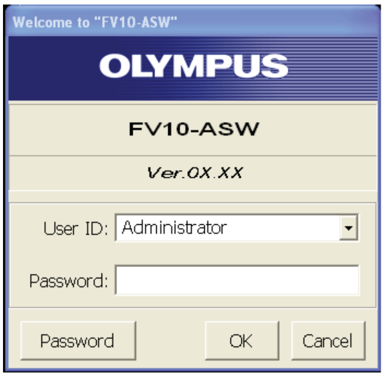
C: MAr laser



D: HeNe (G) laser



5


Welcome to "FV10-ASW"
OLYMPUS
FV10-ASW
Ver.0X.XX
Password
OK
Cancel

User ID: Administrator

Password:

1. Switch on the central power and then start up the Controller.

2. Start up the laser systems.

A: MCPSU (405/440/473/559/635 nm)

Switch on the system.

B: LD559

(1) Switch on the main power of the laser system. The PL lamp lights up in **green** and the TEMP lamp blinks in **green**.

(2) Confirm that the TEMP lamp has changed from blinking to being constantly lit.

(3) Turn on the key switch. The LASER lamps **blink in red**. It takes a while for the lasers to stabilize (The lamps change from **blinking in red** to being constantly **lit in red**). After the LASER lamps are **lit in red**, the LD559 laser system is ready for use.

C: MultiAr laser

Switch on the main power and then turn the key switch clockwise.

D: HeNe (G) laser (543 nm)

Turn the key switch clockwise.

3. Switch on the power of the mercury lamp unit. *** Used for visual observation**

4. Enter the user name and password to log on to Windows.

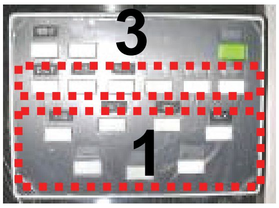
OS	User name	Passwprd
Windows7 (64bit)	olympus	olympus
Others	fluoview	fluoview

5. Double click FV to start up the FV10-ASW software.

User name: Administrator
Password: Administrator

Visual Observation via Microscope

■ ■ Fluorescent image observation ■ ■



Hand switches

■ Memo ■
Fluorescent mirror unit

WBV:	**blue violet excitation (ECFP, etc.)**
NIBA:	**blue excitation/green fluorescence (Example: FITC, EGFP, etc.)**
WIG:	**green excitation/red fluorescence (Example: rhodamine, DsRed, etc.)**
WU:	**UV excitation**

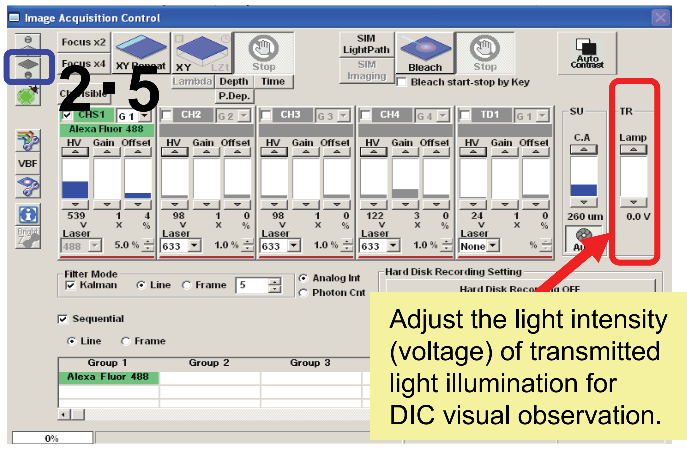


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
P.Dep.
Stop
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
2·5
CH1
CH2
CH3
CH4
TD1
Alexa Fluor 488
HV
Gain
Offset
VBF
SU
TR
C.A
Lamp
260 um
0.0 V
Laser
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Hard Disk Recording Setting
Hard Disk Recording OFF
Sequential
Group 1
Group 2
Group 3
0%

Adjust the light intensity (voltage) of transmitted light illumination for DIC visual observation.

1. Select an objective lens using the hand switches.
2. Click ◆ in the FV10-ASW software.
3. Select a fluorescent mirror unit using the hand switches.
4. Adjust the focus.
5. After observation finishes, click ▣ to close the shutter of the fluorescent lamp.

■ ■ DIC image observation ■ ■

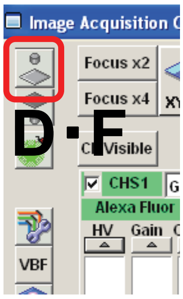
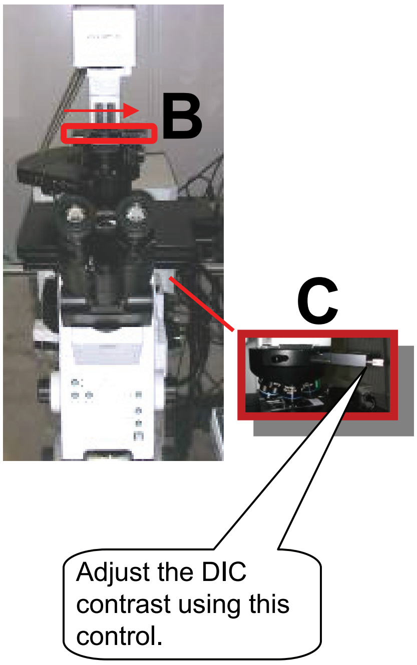


Image Acquisition C
Focus x2
Focus x4
D·F
Visible
CHS1
Alexa Fluor
HV
Gain
VBF



Adjust the DIC contrast using this control.

A. Select an objective lens using the hand switches.

B. Insert the polarizer.

C. Insert the DIC slider.
* Switch the prism position change lever to BFP1 or NORMAL depending on the objective lens used.

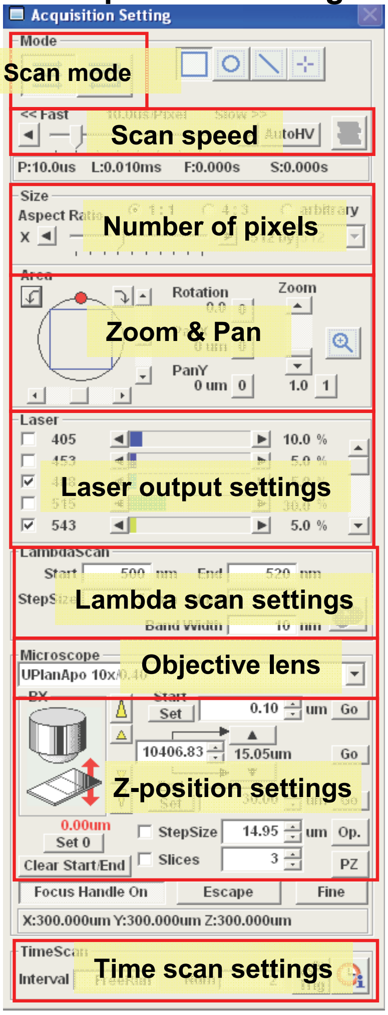
D. Click ◆ in the FV10-ASW software.

E. Adjust the focus.

F. After observation finishes, click ▣ to close the shutter of the halogen lamp.

Overview of Image Acquisition Panel

■ Acquisition Setting ■


Acquisition Setting
Mode
<< Fast
AutoHV
P:10.0us L:0.010ms F:0.000s S:0.000s
Size
Aspect Ratio
Area
Rotation
Zoom
PanY
Laser
405
453
515
543
LambdaScan
Microscope
UPlanApo 10x
Set
Go
0.00um
Set 0
Clear Start/End
StepSize
Slices
Op.
PZ
Focus Handle On
Escape
Fine
X:300.000um Y:300.000um Z:300.000um
TimeScan
Interval

Scan mode

Scan speed

Number of pixels

Zoom & Pan

Laser output settings

Lambda scan settings

Objective lens

Z-position settings

Time scan settings

■ Image Acquisition Control ■

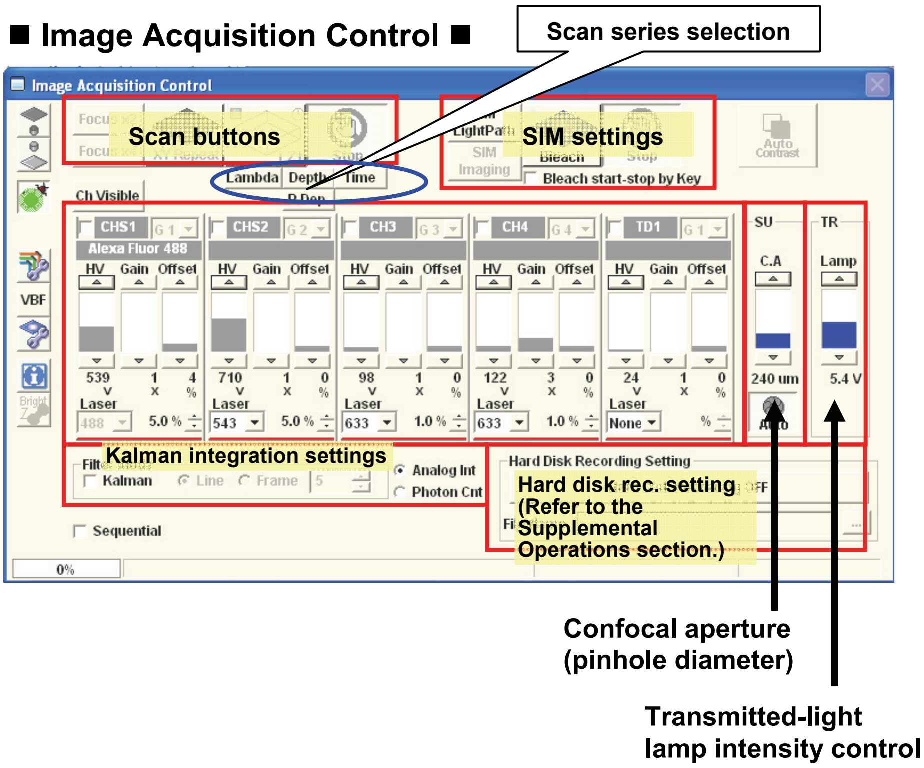


Image Acquisition Control
Lambda
Depth
Time
Bleach start-stop by Key
Ch Visible
VBF
CHS1
CHS2
CH3
CH4
TD1
Alexa Fluor 488
HV
Gain
Offset
Laser
SU
C.A
240 um
TR
Lamp
5.4 V
Auto Contrast
Sequential
Analog Int
Photon Cnt
Kalman
Line
Frame
Hard Disk Recording Setting
0%

Scan series selection

Scan buttons

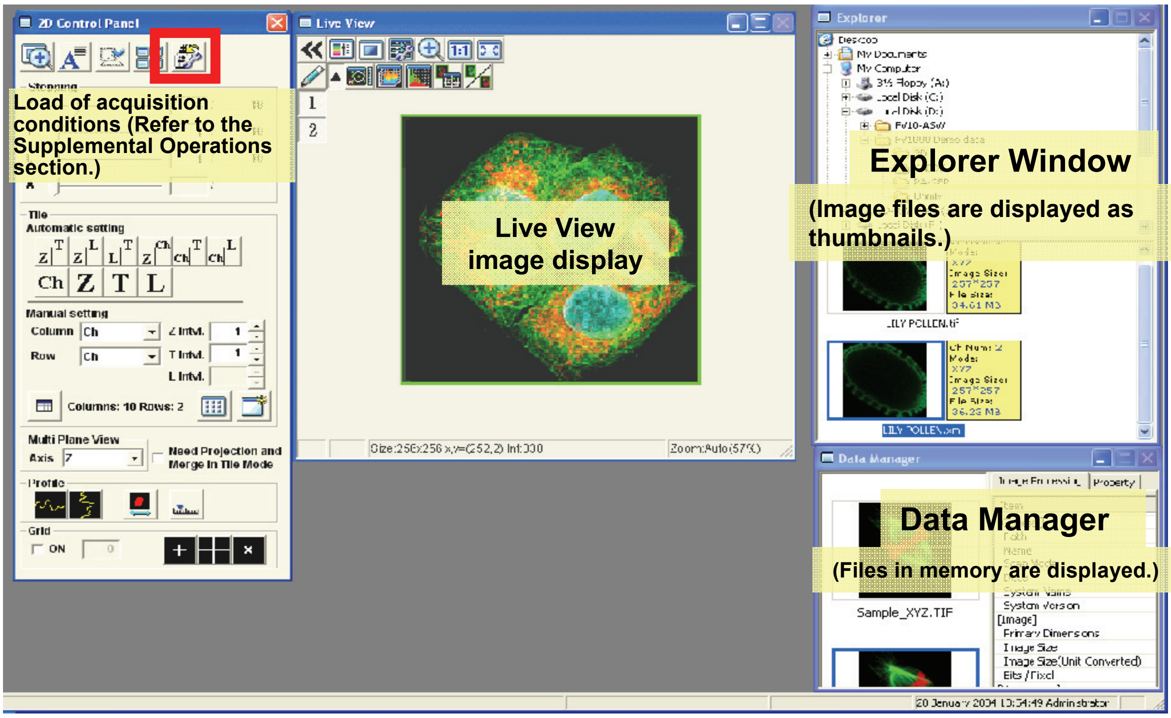
SIM settings

Kalman integration settings

Hard disk rec. setting (Refer to the Supplemental Operations section.)

Confocal aperture (pinhole diameter)

Transmitted-light lamp intensity control


2D Control Panel
Live View
Explorer
Data Manager
Tile
Automatic setting
Manual setting
Columns: 10 Rows: 2
Multi Plane View
Need Projection and Merge In Tile Mode
Profile
Grid
ON
Sample_XYZ.TIF
LILY POLLEN.tif

Load of acquisition conditions (Refer to the Supplemental Operations section.)

Live View image display

Explorer Window

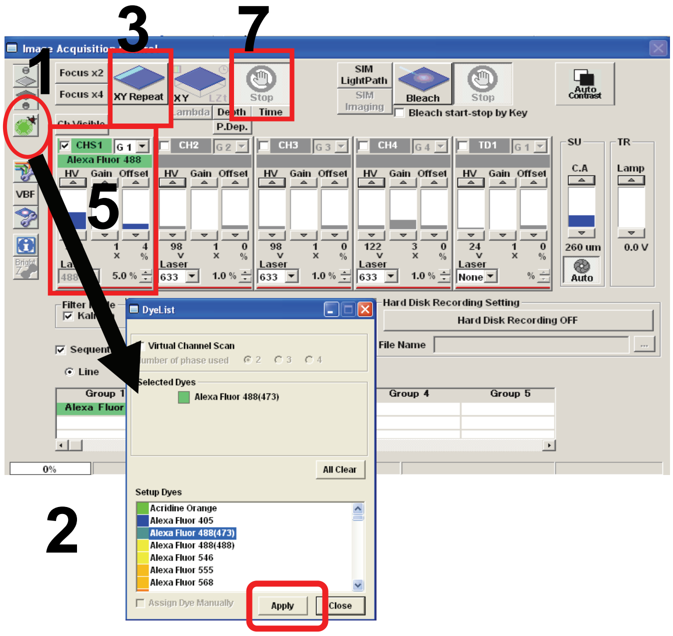
(Image files are displayed as thumbnails.)

Data Manager

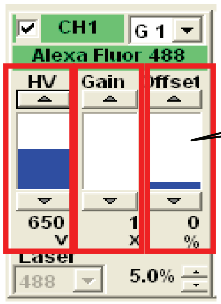
(Files in memory are displayed.)

XY Image Acquisition (Single-color staining)

Example: single-color staining with green fluorescence (Alexa488)


3
7
1
5
2
Image Acquisition
Focus x2
Focus x4
XY Repeat
Stop
SIM LightPath
Bleach
Auto Contrast
CH1
Alexa Fluor 488
CH2
CH3
CH4
TD1
HV
Gain
Offset
Laser
Hard Disk Recording Setting
Hard Disk Recording OFF
File Name
DyeList
Virtual Channel Scan
Selected Dyes
Alexa Fluor 488(473)
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close

5


CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650 V
1 x
0 %
Laser
488
5.0%

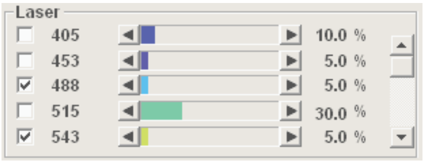
Ch group box adjustment

HV: Adjusts detector sensitivity.

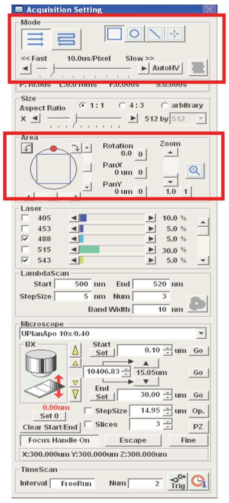
Gain: Brightens images with a set magnification.

Offset: Darkens the background.

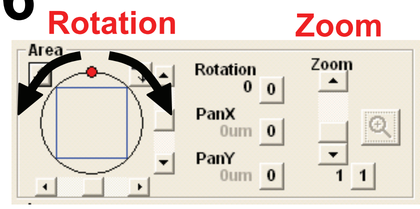
Laser intensity adjustment



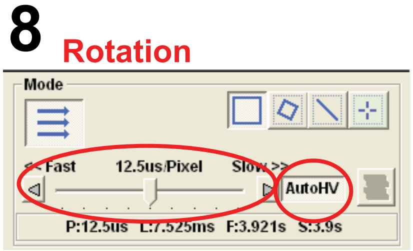
Laser		
☐	405	10.0 %
☐	453	5.0 %
☑	488	5.0 %
☐	515	30.0 %
☑	543	5.0 %


Acquisition Setting
Mode
Size
Area
Laser
LambdaScan
Microscope
TimeScan

6


Rotation
Zoom
Area
Rotation
PanX
PanY
Zoom

8


Rotation
Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us
L:7.525ms
F:3.921s
S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Click the XY Repeat button XY Repeat to execute a continuous scan.

* If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4.

4. Adjust the focus by turning the focus knob.

5. Make adjustments for green imaging (Alexa488) (laser intensity, HV, offset, etc.).

6. Set the zoom and rotation as required.

7. After finishing image adjustment, click the STOP button Stop to stop scanning.

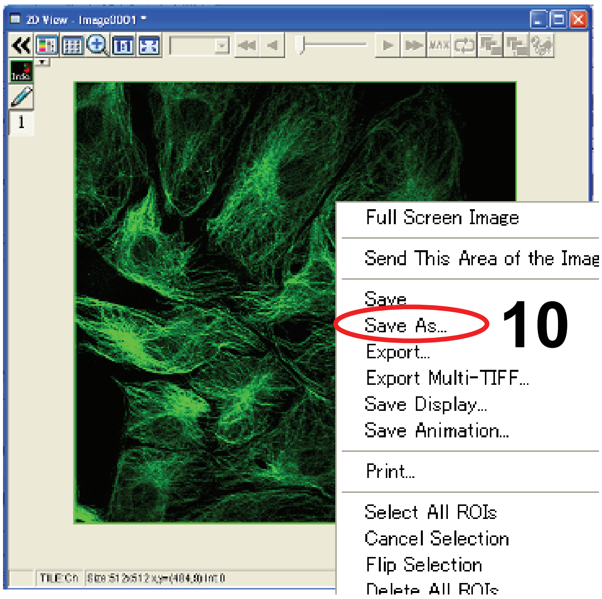
8. Select AutoHV and then select ScanSpeed.

* The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
Alternatively, Kalman integration can be used.
For more information, refer to page 8, "Points on acquiring clear images."

XY Image Acquisition (Single-color staining)

Example: single-color staining with green fluorescence (Alexa488)

9



9. To acquire the image, click the XY button.
10. Save the image: Select **Save As** by right-clicking or going to the File menu, and then save the image in OIB or OIF format.

■ Memo ■
FV10-ASW dedicated file formats

OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an image as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

[Points on acquiring clear images]

For dark images

Increasing the HV: Increases detector sensitivity. Use another method in addition to this if sufficient brightness cannot be obtained even when the HV is increased to 700 V or higher.

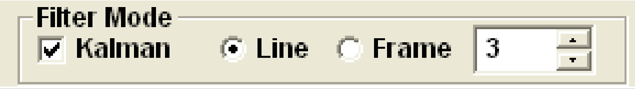
Increasing laser intensity: Increases fluorescent brightness with an increase in excitation light output. Note that an excessively increased intensity may fade fluorescence or cause greater damage to samples.

Increasing the CA: The Auto button stays clicked during regular use so that the optimal pinhole diameter is always automatically set.
Higher brightness can be obtained by deselecting Auto to increase the CA (confocal aperture), which is equivalent to decreasing the Z-axis resolution.

Increasing the gain: Brightens images with the magnification set before image acquisition.

For noisy images

Kalman integration ... Averages images while acquiring as many images as specified. Check the checkbox for Kalman and then select Line (or Frame). Enter **the number of image integrations (the number of scans)**.

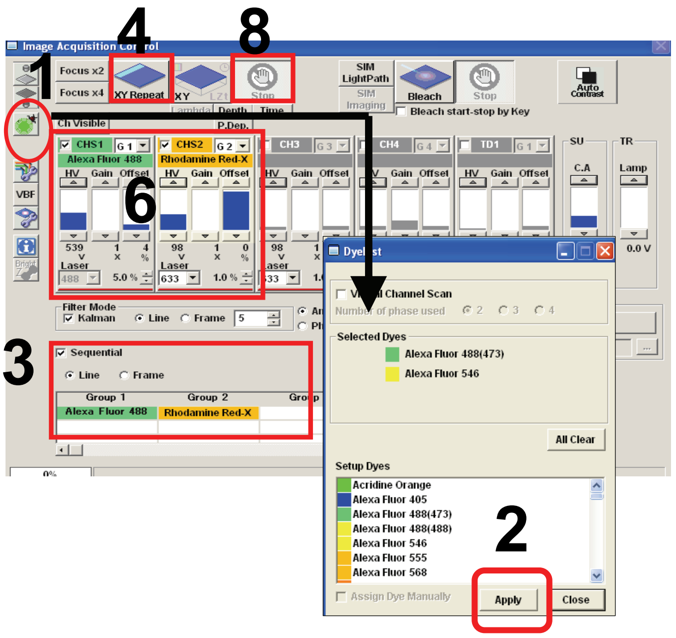


Filter Mode
☑ Kalman ● Line ○ Frame 3

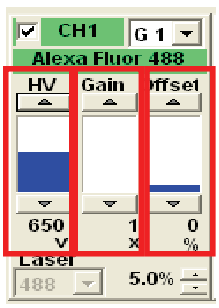
XY Image Acquisition (Multicolor staining)

Line sequential scan

* Mode for avoiding fluorescent crosstalk by individually acquiring fluorescent images


1
4
8
6
3
2
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
SIM LightPath
SIM Imaging
Bleach
Bleach start-stop by Key
Auto Contrast
CH1
Alexa Fluor 488
CH2
Rhodamine Red-X
HV
Gain
Offset
Laser
Filter Mode
Kalman
Line
Frame
Sequential
Group 1
Group 2
Dye List
Virtual Channel Scan
Selected Dyes
Alexa Fluor 488(473)
Alexa Fluor 546
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close

6


CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650 V
1 x
0 %
Laser
488
5.0%

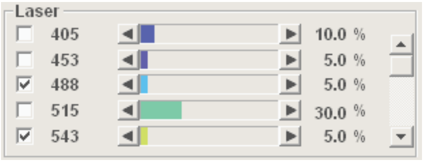
Ch group box adjustment

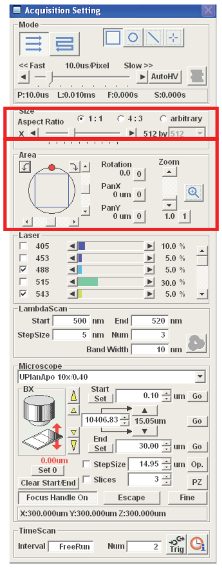
HV: Adjusts detector sensitivity.

Gain: Brightens images with a set magnification.

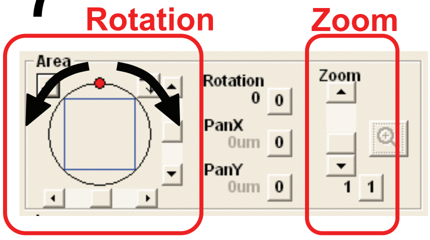
Offset: Darkens the background.

Laser intensity adjustment

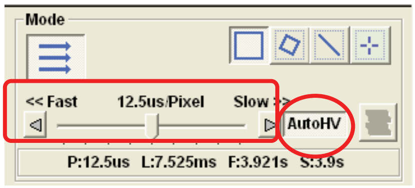

Laser
405
453
488
515
543
10.0 %
5.0 %
5.0 %
30.0 %
5.0 %


Acquisition Setting
Mode
Size
Aspect Ratio
Area
Laser
LambdaScan
Microscope
TimeScan

7


Rotation
Zoom
Area
Rotation
PanX
PanY
0um
1

9


Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us
L:7.525ms
F:3.921s
S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Check the checkbox for Sequential and then select Line.

4. Click the XY Repeat button **XY Repeat** to execute a continuous scan.

   * If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button **Focus x2** **Focus x4** .

5. Adjust the focus by turning the focus knob.

6. Make adjustments for CH1/CH2 imaging (laser intensity, HV, offset, etc.).

7. Set the zoom and rotation as required.

8. After finishing image adjustment, click the STOP button **Stop** to stop scanning.

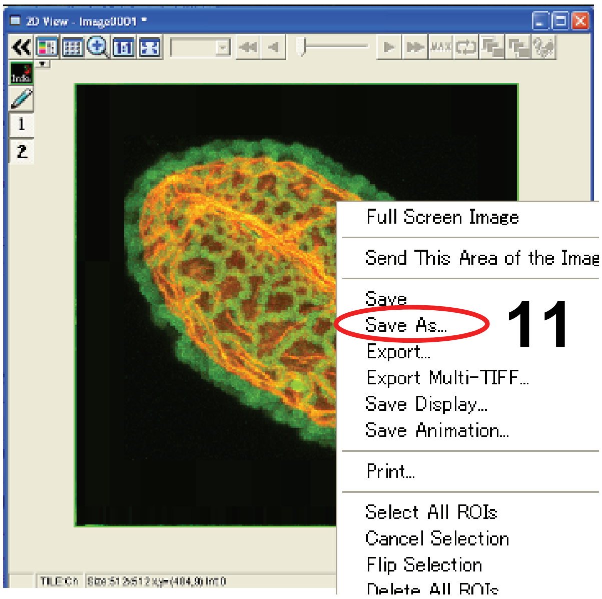
9. Select AutoHV and then select ScanSpeed.

   * The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
   Alternatively, Kalman integration can be used.
   For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Multicolor staining)

Line sequential scan

10


2D View - Image0001
1
2
Full Screen Image
Send This Area of the Imag
Save
Save As...
11
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

10. To acquire the image, click the XY button.
11. Save the image: Select **Save As** by right-clicking or going to the File menu, and then save the image in OIB or OIF format.

■ Memo ■
FV10-ASW dedicated file formats

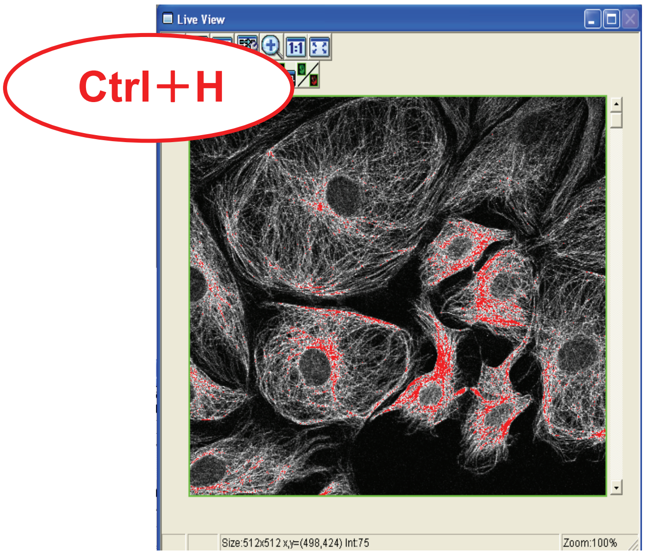
OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

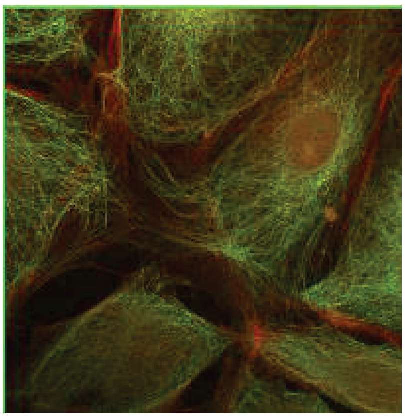
OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an image as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

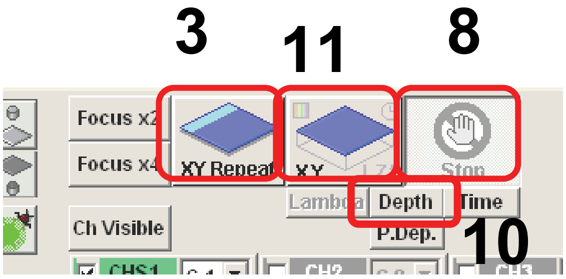
■ Image brightness adjustment ■

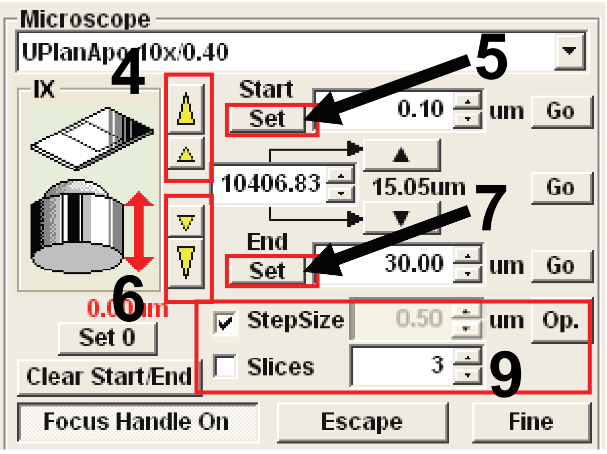
By pressing **Ctrl + H** on the keyboard, an image can be displayed in Hi-Low mode. Pixels of intensity 4095 are shown in red.
To return to normal mode from Hi-Low mode, press **Ctrl + H** again. The image returns to its original color.


Live View
Ctrl＋H
Size:512x512 x,y=(498,424) Int:75
Zoom:100%



XYZ Image Acquisition

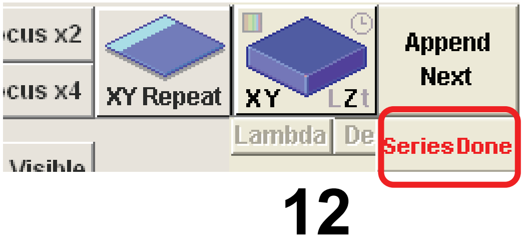

3
11
8
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
P.Dep.
10
Ch Visible
CHS1
CH2
CH3


Microscope
UPlanApo 10x/0.40
4
5
IX
Start
Set
0.10
um
Go
10406.83
15.05um
7
Go
End
Set
30.00
um
Go
6
0.00um
Set 0
StepSize
0.50
um
Op.
Slices
3
9
Clear Start/End
Focus Handle On
Escape
Fine

■ Memo ■

▲ : Each click shifts the position by the value entered for StepSize.

▴ : Each click shifts the position by 1/2 the value entered for StepSize.


cus x2
cus x4
XY Repeat
XY
Z t
Lambda
De
Append Next
Series Done
Visible
12

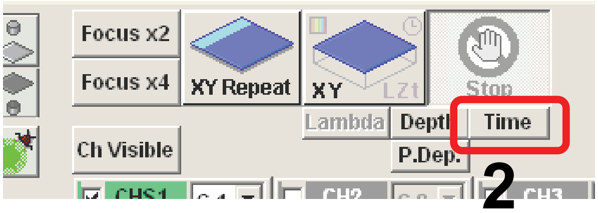
1. Before starting the following procedure, make adjustments for XY imaging.
   (Refer to page 6 or 8.)

The following procedure fixes the upper and lower limit positions of a continuous cross-sectional image.

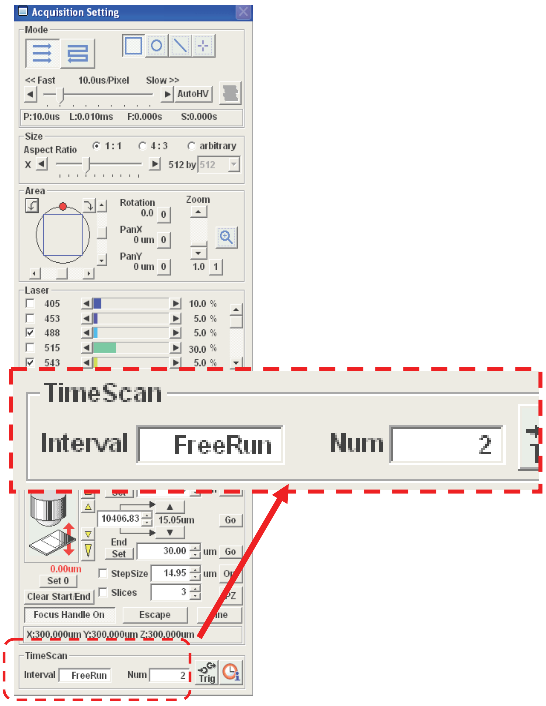
2. Click Clear Start/End to clear the settings of acquisition start and end position. Then, click Set 0 to zero the Z-position values being displayed.
3. Click the XY Repeat button to execute a scan.
4. Click ▲ or ▴ to shift the focus.
   (Refer to ■ Memo 1 ■.)
5. When the upper limit position of the sample is displayed in the image, fix the position by clicking the Set button.
6. Click ▼ or ▾ to shift the focus.
   (Refer to ■ Memo 1 ■.)
7. When the lower limit position of the sample is displayed in the image, fix the position by clicking the Set button.
8. To stop scanning, click the Stop button.
9. Enter a value in StepSize or Slices, and check ☑ the checkbox.
   (Click the Op button to see a recommended value.)
10. Select Depth.
11. To acquire the image, click the XYZ button.
12. To save the image, click Series Done.

For more information on 3D image display, refer to the Supplemental Operations section.

XYT Image Acquisition


Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
P.Dep.
Ch Visible
CHS1
CH2
CH3
2

1. Before starting the following procedure, make adjustments for XY imaging.
   (Refer to page 6 or 8.)
2. Select Time from among the series buttons.

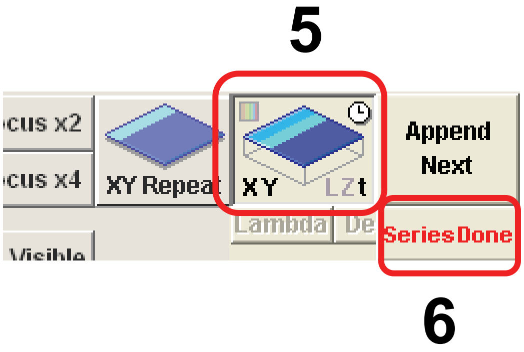

Acquisition Setting
Mode
Size
Area
Laser
TimeScan

TimeScan

Interval FreeRun

Num 2

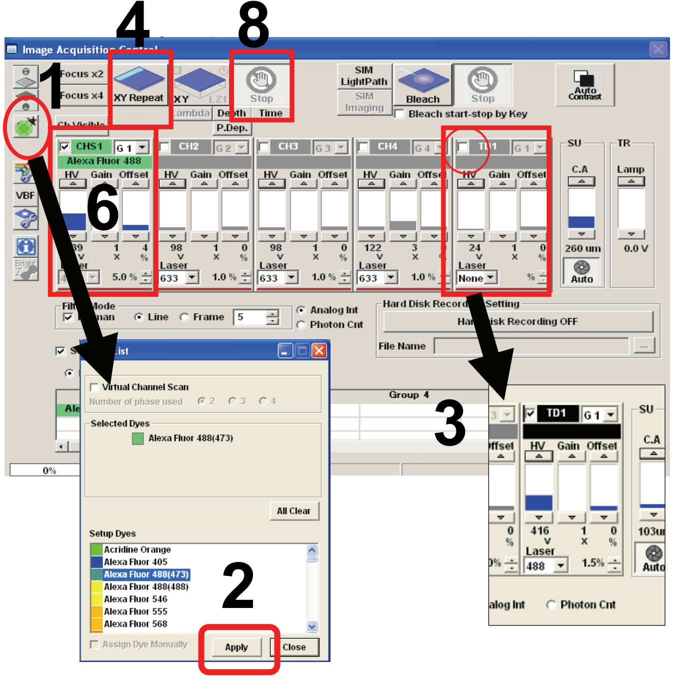
3. In the Interval box, enter the time (in seconds) taken between one acquisition start and the next acquisition start.
   Example: To set a 1 minute interval, type “60” and click Enter.
4. Enter the number of acquisition in the Num box.


5
cus x2
cus x4
XY Repeat
XY
LZt
Append
Next
Lambda
De
Series Done
Visible
6

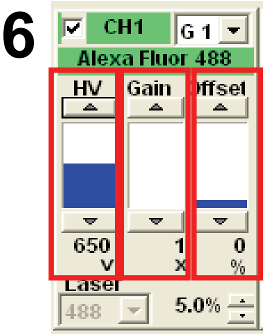
5. To acquire the images, click the XYT button.
6. To save the images, click Series Done.

XY Image Acquisition (Single-color staining + DIC)

Example: green fluorescent + DIC image


4
8
1
6
3
2
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
Stop
CH1
Alexa Fluor 488
HV
Gain
Offset
TD1
Selected Dyes
Alexa Fluor 488(473)
Setup Dyes
All Clear
Apply
Close

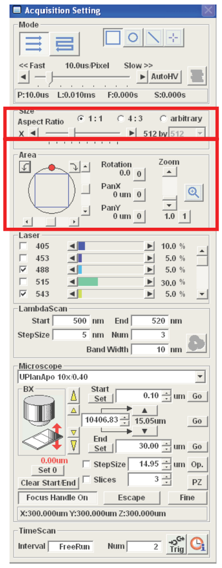
6


CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650
V
1
X
0
%
Laser
488
5.0%

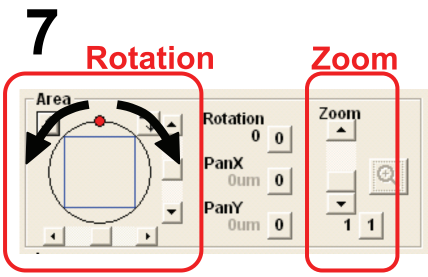
HV: Adjusts detector sensitivity.

Gain: Brightens images with a set magnification.

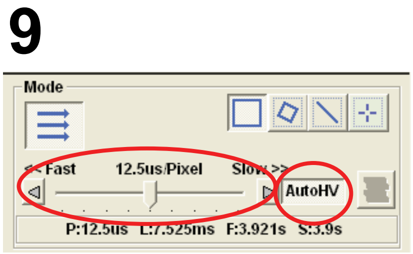
Offset: Darkens the background.


Acquisition Setting
Mode
Size
Area
Laser
LambdaScan
Microscope
TimeScan

7


Rotation
Zoom
Area
Rotation
PanX
PanY
0
1

9


Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us
L:7.525ms
F:3.921s
S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Check the checkbox for TD1.
   Check that the polarizer and the DIC slider for transmitted light are within the optical path limits.

4. Click the XY Repeat button XY Repeat to execute a continuous scan.
   * If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4.

5. Adjust the focus by turning the focus knob.

6. Make adjustments for green imaging (Alexa488) and TD imaging.

7. Set the zoom and rotation as required.

8. After finishing image adjustment, click the STOP button Stop to stop scanning.

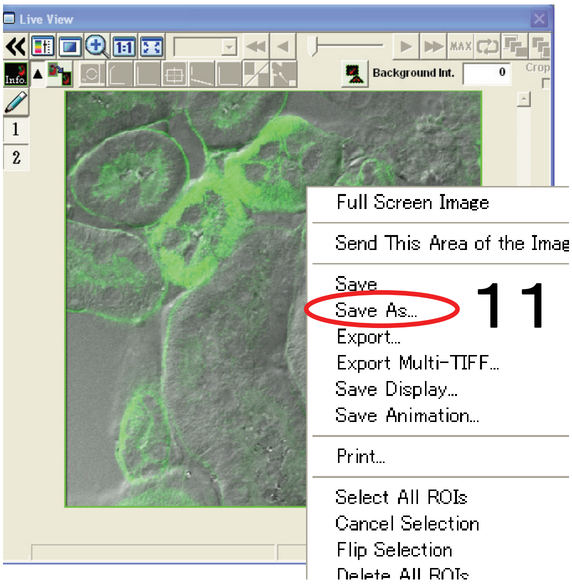
9. Select AutoHV and then select ScanSpeed.

   * The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
   Alternatively, Kalman integration can be used.
   For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Single-color staining + DIC)

Example: green fluorescent + DIC image

10


Live View
Info.
Background Int.
0
Crop
1
2
Full Screen Image
Send This Area of the Imag
Save
Save As...
11
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

10. To acquire the image, click the XY button.

11. Save the image: Select **Save As** by right-clicking or going to the File menu and then save the image in OIB or OIF format.

■ Memo ■
FV10-ASW dedicated file formats

OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

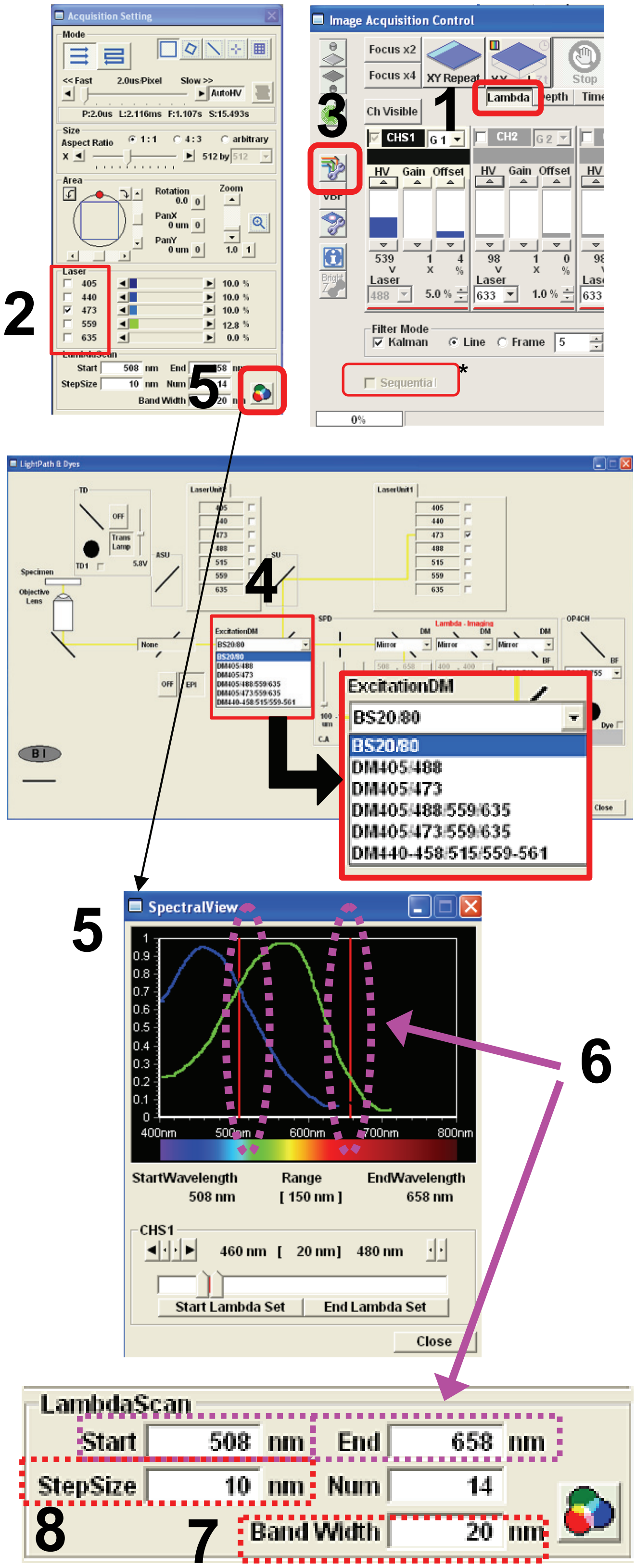
OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an images as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

Only for SPD

XYλ Image Acquisition

Spectral image acquisition


Acquisition Setting
Image Acquisition Control
Lambda
Sequential
LightPath & Dyes
ExcitationDM
BS20/80
DM405/488
DM405/473
DM405/488/559/635
DM405/473/559/635
DM440-458/515/559-561
SpectralView
1
2
3
4
5
6
7
8

StartWavelength 508 nm; Range [150 nm]; EndWavelength 658 nm

CHS1 460 nm [20 nm] 480 nm

LambdaScan

Start 508 nm; End 658 nm

StepSize 10 nm; Num 14

Band Width 20 nm

1. Click **Lambda** .

   * When the Sequential checkbox is checked, the Lambda button is grayed out. Uncheck the checkbox.

2. Check the checkbox for the laser to be used.

3. To display the optical path diagram, click .

4. Select BS20/80 or a DM according to the excitation wavelength.

   Example: Select DM405/473 for excitation at 473.

5. To display SpectralView, click .

6. In the Start and End boxes, enter the wavelength at which to start and end image acquisition.

   These boxes correspond to the red bars in the SpectralView graph.

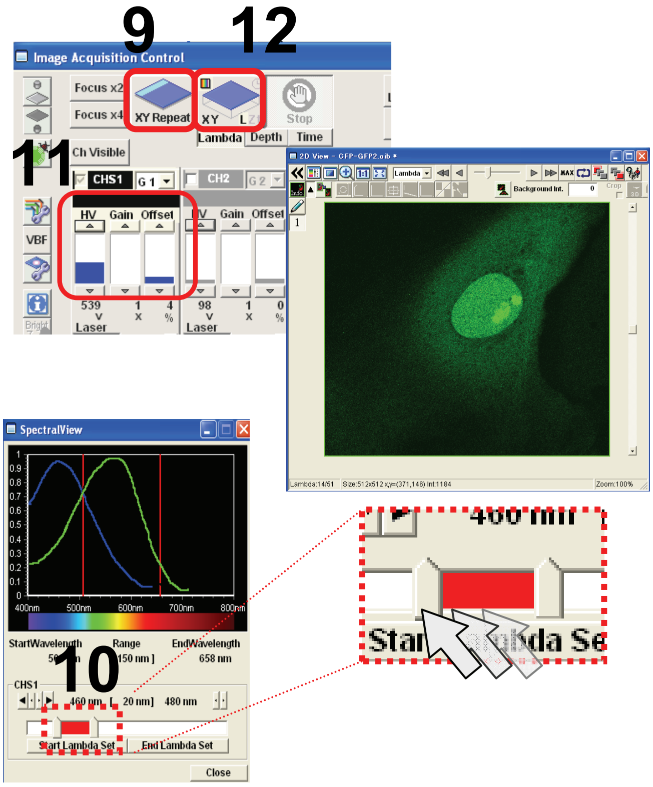
7. In the Band Width box, set the wavelength range of acquisition per frame (Example: 20 nm).

8. In the StepSize box, enter the wavelength interval between one acquisition start and the next acquisition start (Example: 10 nm).

   The minimum settable unit is 1.0 nm.

XYλ Image Acquisition

Spectral image acquisition


9
12
11
10
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
Ch Visible
CHS1
CH2
HV
Gain
Offset
VBF
Laser
2D View - GFP-GFP2.oib
SpectralView
400nm
500nm
600nm
700nm
800nm
StartWavelength
Range
EndWavelength
658 nm
CHS1
460 nm
20 nm
480 nm
Start Lambda Set
End Lambda Set
Close
400 nm

9. To execute a scan, click the XY Repeat button XY Repeat .

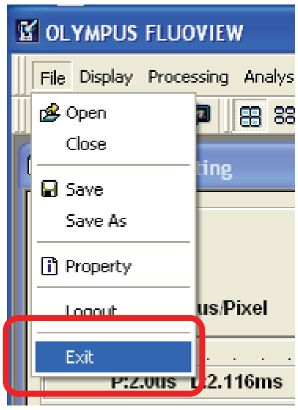
10. While observing the image, find the brightest fluorescent wavelength range by clicking the left slit.

11. Adjust the image.

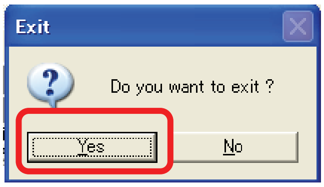
12. To acquire the image, click the XYL button XY LZt .

13. To save the λ image, click SeriesDone SeriesDone .

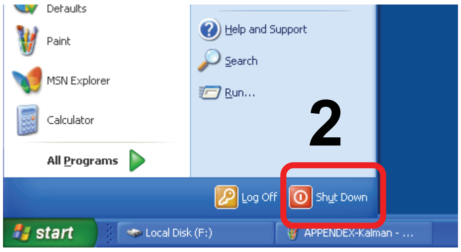
System Shutdown


OLYMPUS FLUOVIEW
File
Display
Processing
Analys
Open
Close
Save
Save As
Property
Logout
Exit
us/Pixel
P:2.0us
2.116ms

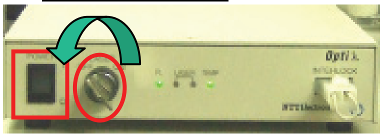
1



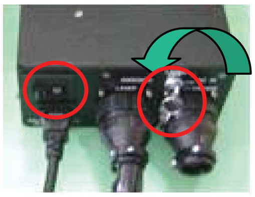
Do you want to exit ?


Defaults
Paint
MSN Explorer
Calculator
All Programs
Help and Support
Search
Run...
2
Log Off
Shut Down
start
Local Disk (F:)
APPENDEX-Kalman - ...

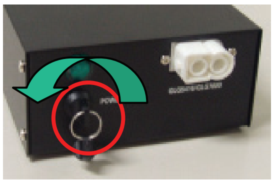
3 **B: LD559nm**

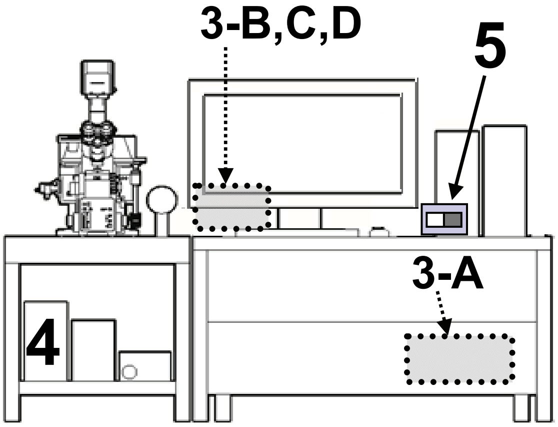


C: MAr laser



D: HeNe (G) laser





1. Exit the FV10-ASW software by selecting Exit from the File menu.

2. Shut down Windows.

3. Turn off the laser systems.

   A: MCPSU (405/440/473/635 nm lasers)
   Switch off the system.

   B: LD559nm
   Turn off the key switch to turn off the main power.

   C: Multi Ar laser
   Turn off the key switch to turn off the main power.

   D: HeNe (G) laser (543 nm)
   Turn off the key switch.

4. Switch off the power of the mercury lamp.

5. Switch off the central power.

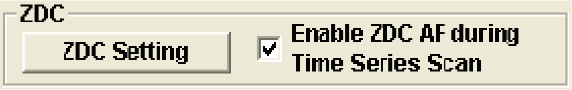
Image Acquisition

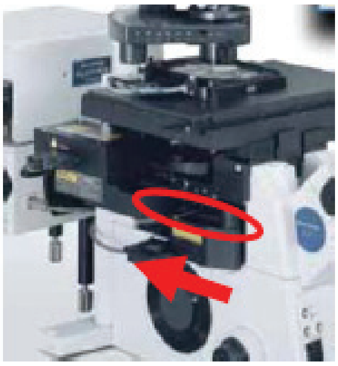
— Applications —

Only for ZDC

XYT Image Acquisition – using ZDC –

1. Check the checkbox for “Enable ZDC AF during Time Series Scan” in the ZDC group box and then click the ZDC Setting button to open the ZDC Control window.


ZDC
ZDC Setting
☑ Enable ZDC AF during Time Series Scan



2. With an objective lens capable of ZDC control, put the ZDC into the optical path.

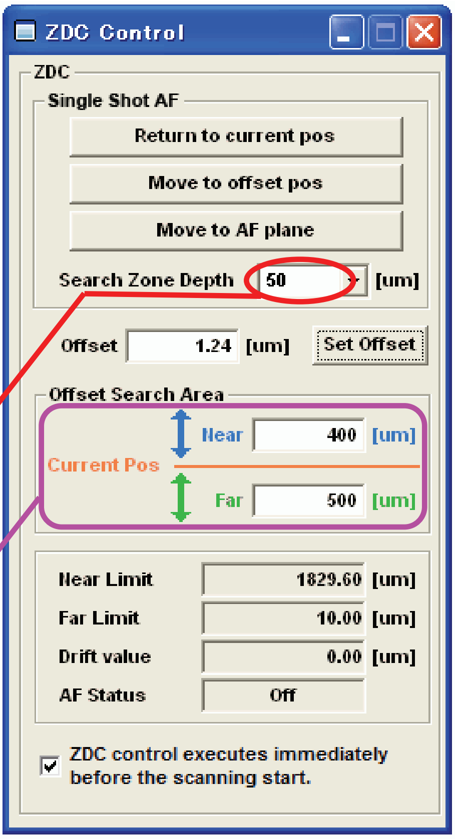
3. Set the image acquisition mode to XYT or XYZT. Adjust the focus on the sample and make image adjustments.

4. Click the ZDC Setting button in the Acquisition Setting window.

5. Click the Set Offset button in the ZDC Control window and set the focus offset value*.

*** The “focus offset value” sets the distance between the glass surface, on which the ZDC is detected, and the focus position of the sample.**

6. Start XYT or XYZT image acquisition. ZDC control is carried out at each interval when T series image acquisition is executed.



Search Zone Depth 50 [um]

Offset 1.24 [um]

Near 400 [um]

Far 500 [um]

Near Limit 1829.60 [um]

Far Limit 10.00 [um]

Drift value 0.00 [um]

AF Status Off

☑ ZDC control executes immediately before the scanning start.

After starting, it searches for an AF position within ±25 μm from the previous AF position.

Sets an area in which to search for an offset.

Notes
ZDC control can be used with non-FreeRUN intervals that are 10 seconds or longer, and the checkbox for “ZDC control executes immediately before the scanning start” in the ZDC group box is checked.
When ZDC control image acquisition is successful, the buzzer issues one beep.
When ZDC control fails, the buzzer issues three beeps.

XYT Image Acquisition
(Photobleaching with a single scanner)

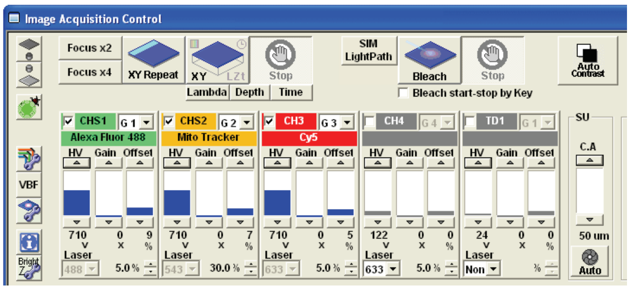
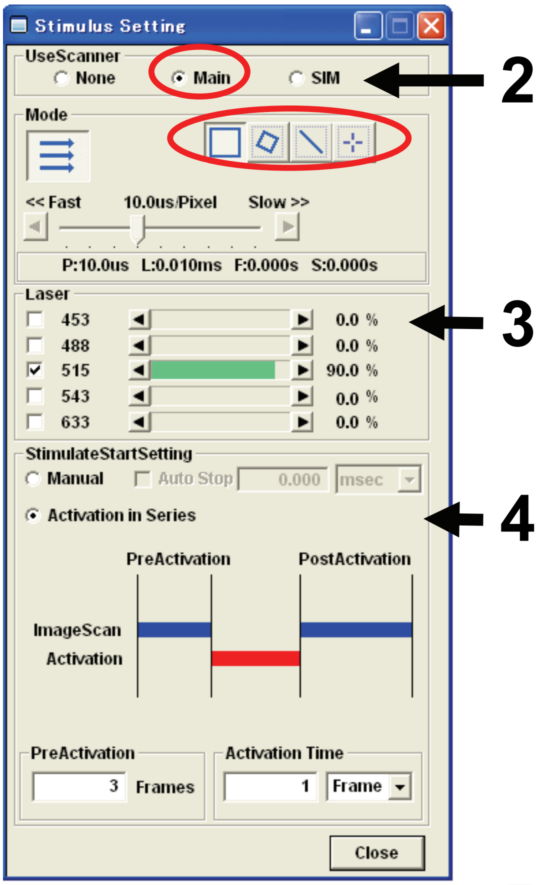


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
VBF
Bright
CHS1
Alexa Fluor 488
CHS2
Mito Tracker
CH3
Cy5
CH4
TD1
HV
Gain
Offset
Laser
SU
C.A
50 um
Auto

1. Make imaging adjustments.


Stimulus Setting
2
3
4
ImageScan
Activation
Close

UseScanner: ○ None ● Main ○ SIM

Mode

<< Fast 10.0us/Pixel Slow >>

P:10.0us L:0.010ms F:0.000s S:0.000s

Laser
☐ 453 0.0 %
☐ 488 0.0 %
☑ 515 90.0 %
☐ 543 0.0 %
☐ 633 0.0 %

StimulateStartSetting
○ Manual ☐ Auto Stop 0.000 msec
● Activation in Series

PreActivation PostActivation

PreActivation: 3 Frames

Activation Time: 1 Frame

2. Select Main in the Use Scanner window. Enclose the region to be photobleached using controls in the Mode section.

3. In the Laser section, select the photobleaching power.

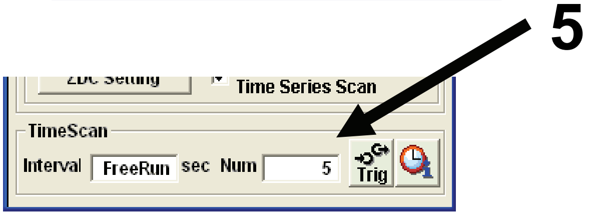
4. Set StimulateStartSetting.

When linking photobleaching with imaging

4-1. Click the radio button for Activation in Series.

4-2. Enter the PreActivation and Activation Time fields.

(For manual operation, refer to the following page.)


5
ZDC Setting
Time Series Scan
Trig

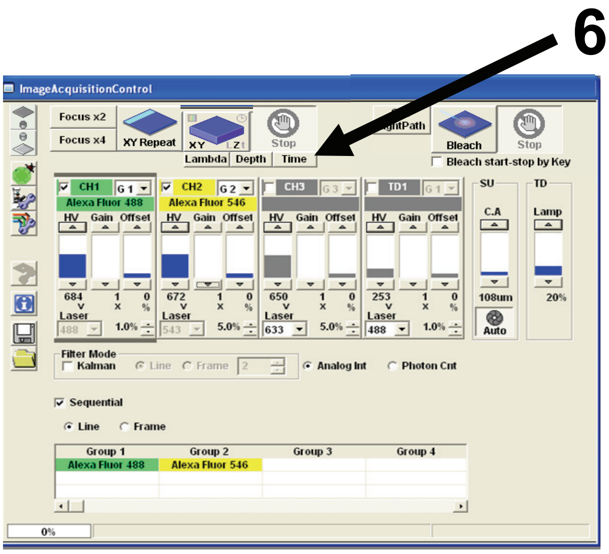
TimeScan

Interval FreeRun sec Num 5

5. Enter the time interval and number.

XYT Image Acquisition

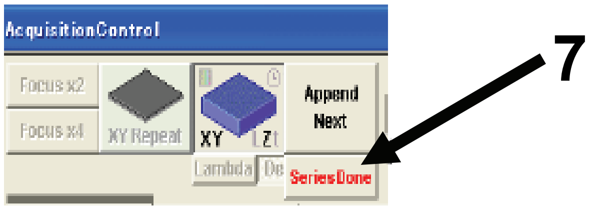
(Photobleaching with a single scanner)


6
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
LightPath
Bleach
Stop
Bleach start-stop by Key
CH1
Alexa Fluor 488
CH2
Alexa Fluor 546
CH3
TD1
SU
TD
Filter Mode
Kalman
Analog Int
Photon Cnt
Sequential
Line
Frame
Group 1
Group 2
Group 3
Group 4

6. With Time selected, click the XYT button to execute image acquisition.

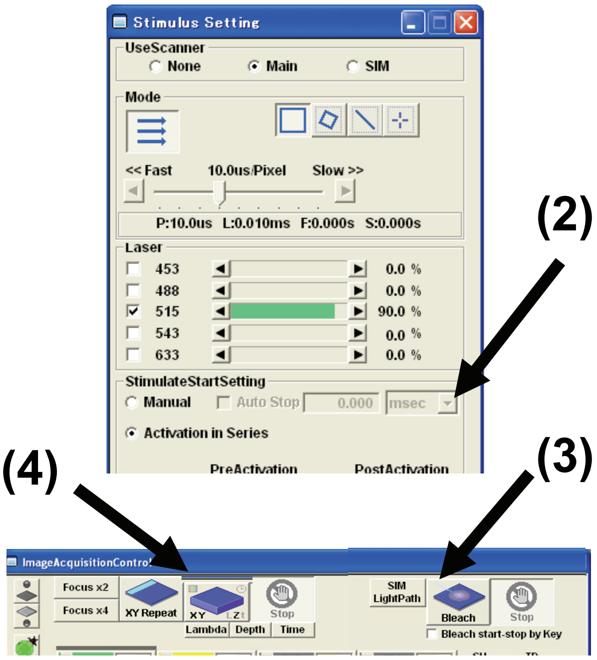
When linking photobleaching with imaging

The region is automatically photobleached at a set time.


7
AcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Append
Next
SeriesDone

7. Clicking SeriesDone causes the window bar of the acquired image to show “2DView-LiveImage(x).”

8. Save the image:
   Save the image by selecting Save As from File on the menu bar.
   (The Save As types “oib” and “oif” are the dedicated file formats for the FV10-ASW software.)


Stimulus Setting
UseScanner
None
Main
SIM
Mode
<< Fast
10.0us/Pixel
Slow >>
P:10.0us L:0.010ms F:0.000s S:0.000s
Laser
453
488
515
543
633
StimulateStartSetting
Manual
Auto Stop
msec
Activation in Series
PreActivation
PostActivation
(2)
(3)
(4)
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
SIM LightPath
Bleach
Stop
Bleach start-stop by Key

When photobleaching manually

(1) Perform operations 1 to 3 on the previous page.

(2) Click the radio button for Manual.
(To set the amount of time the Bleach button is enabled when it is clicked, check the checkbox for Auto Stop and enter the number of seconds.)

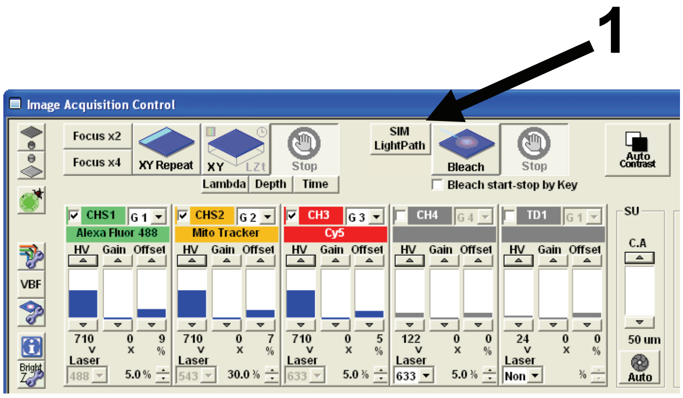
(3) Click the Bleach button.

(4) Observe the image in XY Repeat, XY, or XYT.

Only for SIM

XYT Image Acquisition

(Photobleaching with a SIM scanner)


1
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
VBF
CHS1
Alexa Fluor 488
CHS2
Mito Tracker
CH3
Cy5
CH4
TD1
HV
Gain
Offset
Laser
SU
C.A
50 um
Auto

CHS1 ☑ G1, Alexa Fluor 488: HV 710, Gain 0, Offset 9; Laser 488, 5.0 %

CHS2 ☑ G2, Mito Tracker: HV 710, Gain 0, Offset 7; Laser 543, 30.0 %

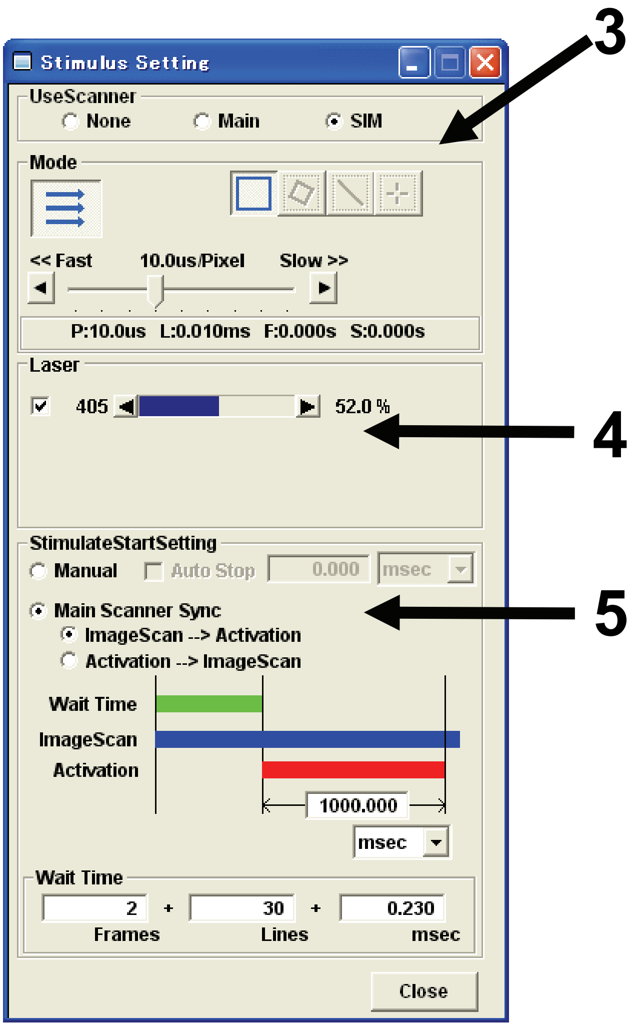
CH3 ☑ G3, Cy5: HV 710, Gain 0, Offset 5; Laser 633, 5.0 %

CH4 ☐ G4: HV 122, Gain 0, Offset 0; Laser 633, 5.0 %

TD1 ☐ G1: HV 24, Gain 0, Offset 0; Laser Non

1. Click SIM LightPath.

2. Make imaging adjustments.


3
4
5
Stimulus Setting
UseScanner
Mode
<< Fast
Slow >>
Laser
StimulateStartSetting
Wait Time
ImageScan
Activation
Close

UseScanner: ○ None ○ Main ● SIM

Mode: 10.0us/Pixel

P:10.0us L:0.010ms F:0.000s S:0.000s

Laser: ☑ 405 52.0 %

StimulateStartSetting: ○ Manual ☐ Auto Stop 0.000 msec

● Main Scanner Sync

● ImageScan --> Activation

○ Activation --> ImageScan

1000.000 msec

Wait Time

2 + 30 + 0.230

Frames Lines msec

3. Select SIM in the Use Scanner window. Enclose the region to be photobleached using controls in the Mode section.

4. In the Laser section, select the photobleaching power.

5. Set StimulateStartSetting.

When photobleaching manually

5-1. Click the radio button for Manual.

(To set the amount of time the Bleach button is enabled when it is clicked, check the checkbox for Auto Stop and enter the number of seconds.)

When linking photobleaching with imaging

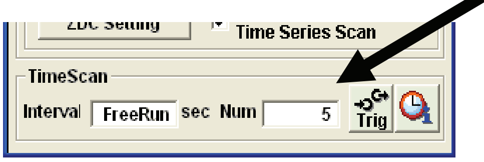
5-1. Click the radio button for Main Scanner Sync and for ImageScan → Activation.

5-2. Enter the values for Activation and Wait Time.

XYT Image Acquisition

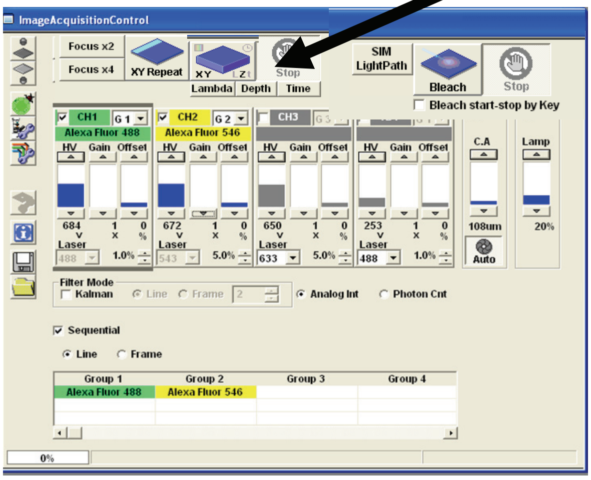
(Photobleaching with a SIM scanner)

6


ZDC Setting
Time Series Scan
TimeScan
Interval
FreeRun
sec
Num
5
Trig

6. Enter the time interval and number.

7


ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Zt
Lambda
Depth
Time
Stop
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
CH1
Alexa Fluor 488
CH2
Alexa Fluor 546
CH3
HV
Gain
Offset
Laser
C.A
Lamp
Auto
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Sequential
Line
Frame
Group 1
Group 2
Group 3
Group 4
Alexa Fluor 488
Alexa Fluor 546
0%

7. With Time selected, click the XYT button to execute image acquisition.

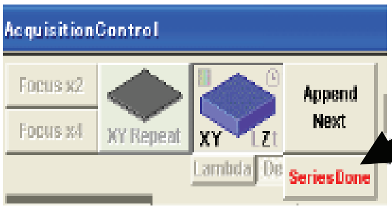
   When photobleaching manually

   Click the Bleach button when you want to start photobleaching.

   When linking photobleaching with imaging

   The region is automatically photobleached at the set time.

8


AcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Zt
Lambda
Append
Next
SeriesDone

8. Clicking SeriesDone causes the window bar of the acquired image to show “2DView-LiveImage(x).”

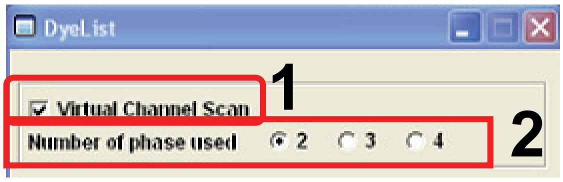
9. Save the image:
   Save the image by selecting Save As from File on the menu bar.
   (The Save As types “oib” and “oif” are the dedicated file formats for the FV10-ASW software.)

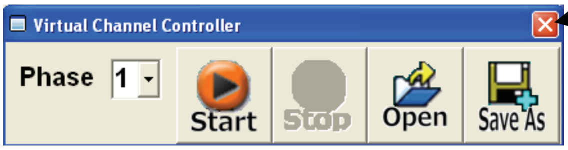
XY Image Acquisition

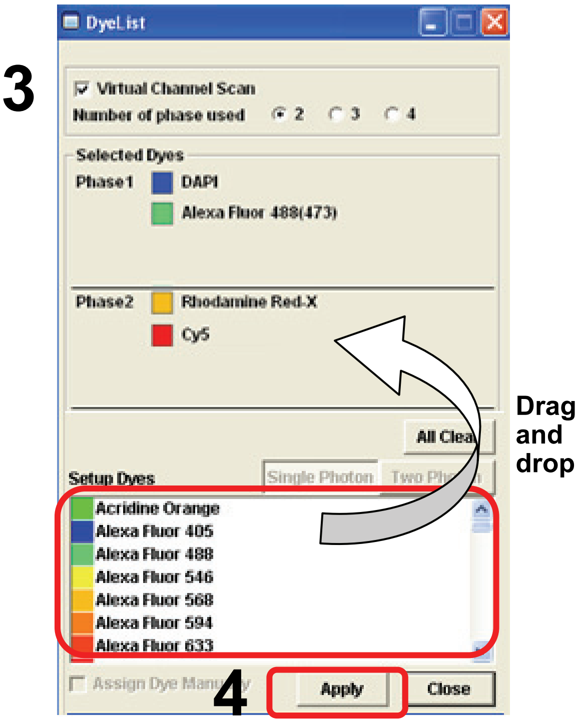
(Four-color staining with virtual channel)

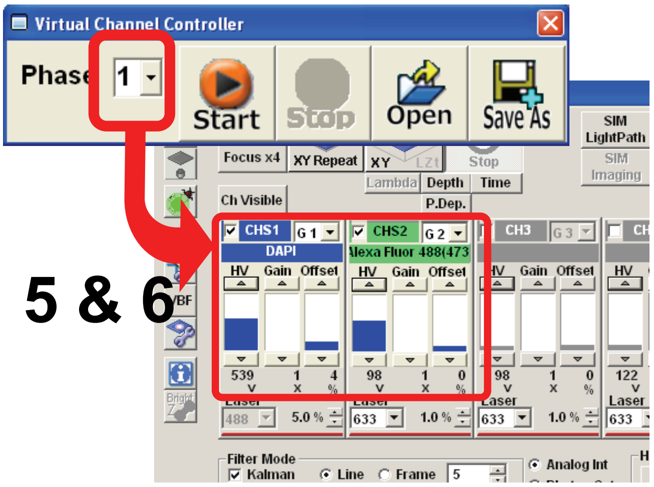
Acquiring a four-color staining image with virtual channel scan

Example: DAPI and Alexa488 for phase 1 / RodaminRed and Cy5 for phase 2


DyeList
☑ Virtual Channel Scan
1
Number of phase used ● 2 ○ 3 ○ 4
2


Virtual Channel Controller
Phase 1
Start
Stop
Open
Save As


3
DyeList
☑ Virtual Channel Scan
Number of phase used ● 2 ○ 3 ○ 4
Selected Dyes
Phase1 DAPI
Alexa Fluor 488(473)
Phase2 Rhodamine Red-X
Cy5
All Clear
Drag and drop
Setup Dyes
Single Photon
Two Photon
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488
Alexa Fluor 546
Alexa Fluor 568
Alexa Fluor 594
Alexa Fluor 633
☐ Assign Dye Manually
4
Apply
Close


5 & 6
Virtual Channel Controller
Phase 1
Start
Stop
Open
Save As
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
P.Dep.
Ch Visible
SIM LightPath
SIM Imaging
CHS1 G1
DAPI
CHS2 G2
Alexa Fluor 488(473)
CH3 G3
HV Gain Offset
539
98
122
Laser
488
633
5.0 %
1.0 %
Filter Mode
☑ Kalman
● Line ○ Frame 5
● Analog Int

1. Check the checkbox for ☑ Virtual Channel Scan in DyeList to automatically open the Virtual Channel Controller.

2. In “Number of phase used,” select “2” as the phase count.

3. Drag a dye from the dye list to each Phase.

4. To set the fluorescent dyes and the phase count, click Apply.

5. Select Phase1 to display DAPI and Alexa488 in Image Acquisition Control.

   *** The slit width, filter, and DM settings are switched to DAPI and Alexa488.**

   Adjust the image.

6. Select Phase2 and adjust the image again.

7. To acquire the image, click Virtual Channel Controller Start .

8. Save the image.

The use of this function does not reflect the LUT setting.

After image acquisition finishes, change the LUT setting by using LUT Setting .

HDRi Image Acquisition

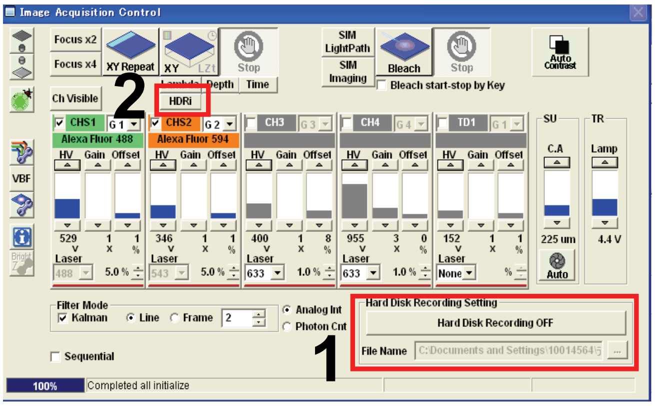


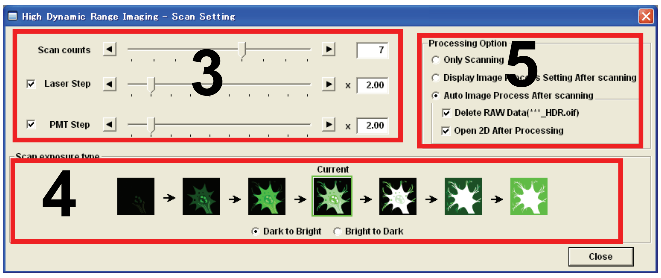
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
SIM LightPath
SIM Imaging
Bleach
Stop
Auto Contrast
Ch Visible
VBF
2
HDRi
CHS1
G 1
Alexa Fluor 488
CHS2
G 2
Alexa Fluor 594
CH3
G 3
CH4
G 4
TD1
G 1
SU
TR
C.A
Lamp
HV
Gain
Offset
Laser
Auto
1

CHS1: HV 529 V, Gain 1 X, Offset 1 %, Laser 488, 5.0 %
CHS2: HV 346 V, Gain 1 X, Offset 1 %, Laser 543, 5.0 %
CH3: HV 400 V, Gain 1 X, Offset 8 %, Laser 633, 1.0 %
CH4: HV 955 V, Gain 3 X, Offset 0 %, Laser 633, 1.0 %
TD1: HV 152 V, Gain 1 X, Offset 1 %, Laser None
C.A 225 um
Lamp 4.4 V

Filter Mode: ☑ Kalman ● Line ○ Frame 2
● Analog Int ○ Photon Cnt
☐ Sequential

Hard Disk Recording Setting: Hard Disk Recording OFF
File Name: C:\Documents and Settings\10014564\

100% Completed all initialize


High Dynamic Range Imaging - Scan Setting
3
5
Scan exposure type
4
Current
Close

Scan counts: 7
☑ Laser Step: x 2.00
☑ PMT Step: x 2.00

Processing Option:
○ Only Scanning
○ Display Image Process Setting After scanning
● Auto Image Process After scanning
☑ Delete RAW Data(***_HDR.oif)
☑ Open 2D After Processing

● Dark to Bright ○ Bright to Dark

HDRI Scan Setting window

1. After finishing image adjustment, set the save destination for hard disk recording.

2. Turn on HDRi in the Image Acquisition Control to open the HDRI Scan Setting window.

3. Set the condition for HDRi.
   - Scan count: number of scans (2 to 10)
   - Laser Step: increase/decrease rate of laser output
   - PMT Step: increase/decrease rate of HV

   *** The value of laser output in Acquisition Setting and the value of HV in Image Acquisition Control are applied to the basic values for HDRi.**

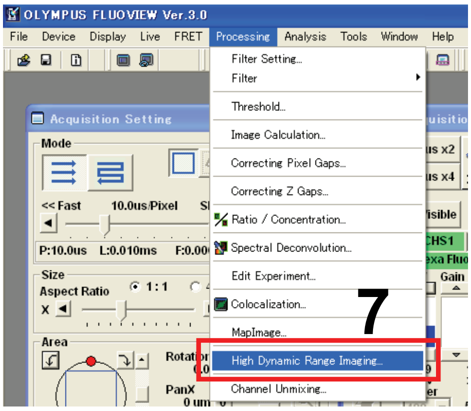
4. Select “Dark to Bright” or “Bright to Dark” and click the icon of acquisition sequence which is given the basic values for HDRi. “Current” is shown on the top of the selected icon.

Example of settings

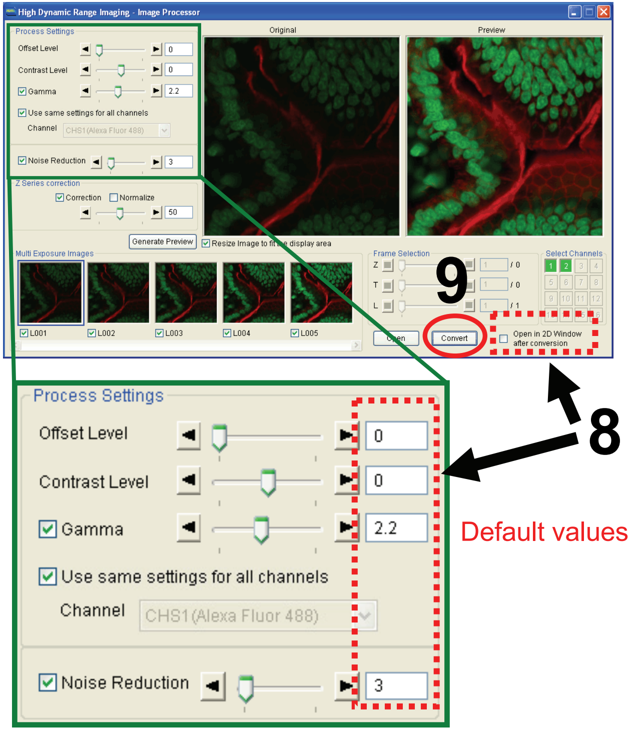
<Basic values>
Laser output in Acquisition Setting: 10.0 (%)
HV in Image Acquisition Control: 500 (V)
<Condition settings for HDRi >
Scan count = 5, Laser Step = x1.2, PMT Step = x1.2,
Dark to Bright, “Current” icon = 3rd from the left
<Setting results>
Laser output (%) : 6.9 -> 8.3 -> 10.0 -> 12.0 -> 14.4
HV (V) : 347 -> 417 -> 500 -> 600 -> 720

5. In the Processing Option section, set the process to be executed after image acquisition finishes.
   - **Only Scanning**: Only executes scanning.
   - **Display Image Process Setting After scanning**: Displays the HDRi Image Processor window after scanning finishes.
   - **Auto Image Process After scanning**: Executes HDRi image processing automatically after scanning finishes. (The parameters used when HDRi Image Processor was last used are applied.)

HDRi Image Acquisition


OLYMPUS FLUOVIEW Ver.3.0
File
Device
Display
Live
FRET
Processing
Analysis
Tools
Window
Help
Acquisition Setting
Filter Setting...
Filter
Threshold...
Image Calculation...
Correcting Pixel Gaps...
Correcting Z Gaps...
Ratio / Concentration...
Spectral Deconvolution...
Edit Experiment...
Colocalization...
MapImage...
High Dynamic Range Imaging...
Channel Unmixing...
7

HDRi Image Processor window


High Dynamic Range Imaging - Image Processor
Original
Preview
Z Series correction
Correction
Normalize
Generate Preview
Resize Image to fit the display area
Multi Exposure Images
L001
L002
L003
L004
L005
Frame Selection
Select Channels
Open
Convert
Open in 2D Window after conversion
9
8
Default values

Process Settings
Offset Level 0
Contrast Level 0
☑ Gamma 2.2
☑ Use same settings for all channels
Channel CHS1 (Alexa Fluor 488)
☑ Noise Reduction 3

Offset Level: The intensity less then Offset value is regarded as 0. The low intensity noise on the image can be eliminated.
Contrast Level: The grayscale of entire images can be adjusted.
Gamma: Gamma correction.
Use same settings for all channels: If it is ticked, the same settings are performed in the image processing of all channels.
Noise Reduction: The noise can be reduced without losing the characteristics of the sample.

6. To start scanning images, click the XY button. Scanned images are saved in the set save destination for hard disk recording.
   (File names are assigned with the format ***_**HDR.oif**.)

When Auto Image Process After scanning is selected in step 6, images are saved after being automatically formatted in HDRI (Files named ***_.oif).

7. From the menu, select Processing and then High Dynamic Range Image to open the HDRi Image Processor window.

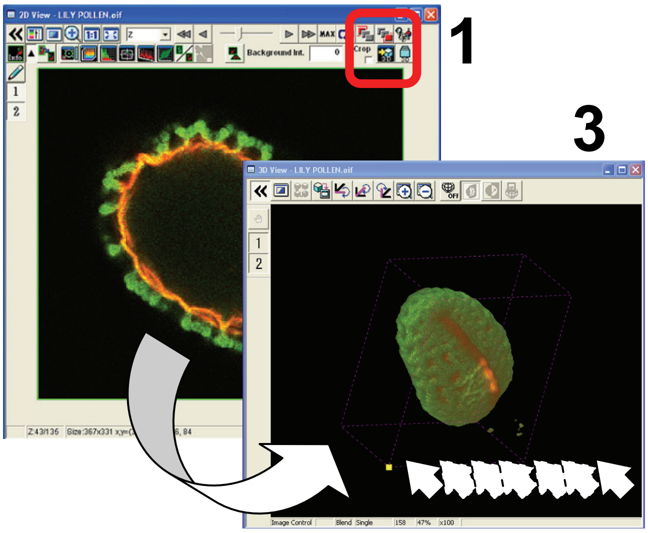
When Display Image Process Setting After scanning is selected in step 6, the window automatically opens.

8. Set the Offset Level, Contrast Level, Gamma and Noise Reduction, and then check the checkbox of "Open in 2D Window after conversion".

9. To create HDRi images, click the Convert button.

— Supplemental Operations —

3D Image Analysis 3D image creation/cross-sectional observation

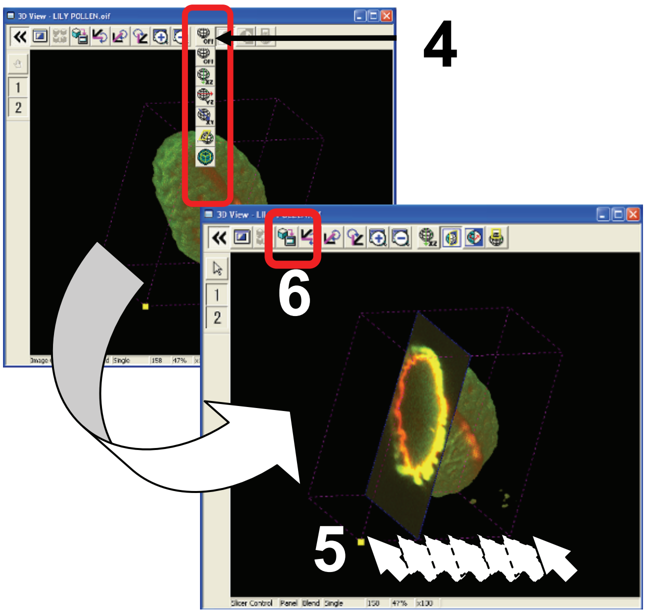

2D View - LILY POLLEN.oif
1
3
3D View - LILY POLLEN.oif

Observe an image from a desired angle.

1. Click 3D of the 2D View-(file name) image.

2. A 3D View is created.

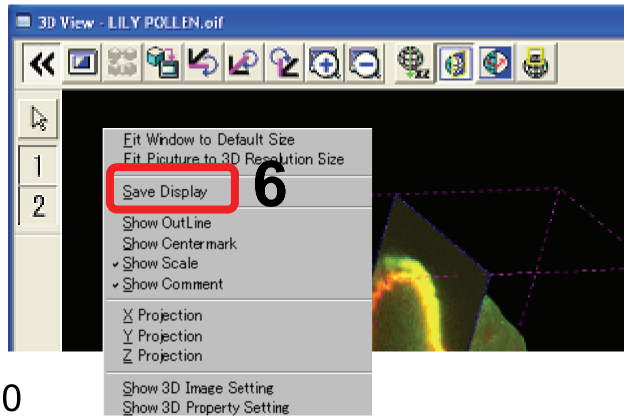
3. To observe the object from a desired angle, move the 3D object by dragging the mouse on the image.


3D View - LILY POLLEN.oif
4
6
5

Observe the desired cross-section of an image.

4. Click OFF and then select XZ.

5. To observe the desired vertical cross-section, move the object right and left by dragging the mouse on the image.


3D View - LILY POLLEN.oif
Fit Window to Default Size
Fit Picuture to 3D Resolution Size
Save Display
6
Show OutLine
Show Centermark
Show Scale
Show Comment
X Projection
Y Projection
Z Projection
Show 3D Image Setting
Show 3D Property Setting

Save the 3D image.

6. Click to create a 2D image. Save the image by right-clicking.

(Or, right click on the 3D image in order to select Save Display to save the view, including the ROI being displayed, in BMP format.)

3D Image Analysis

Detailed settings for 3D image creation

Cut View button
Projects a cross-sectional view on a slice when the slice graphics mode is set.

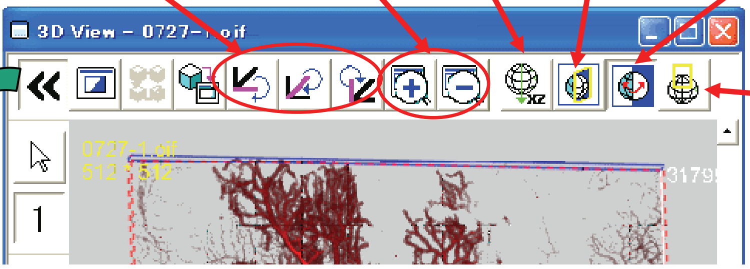
Image zoom in/out

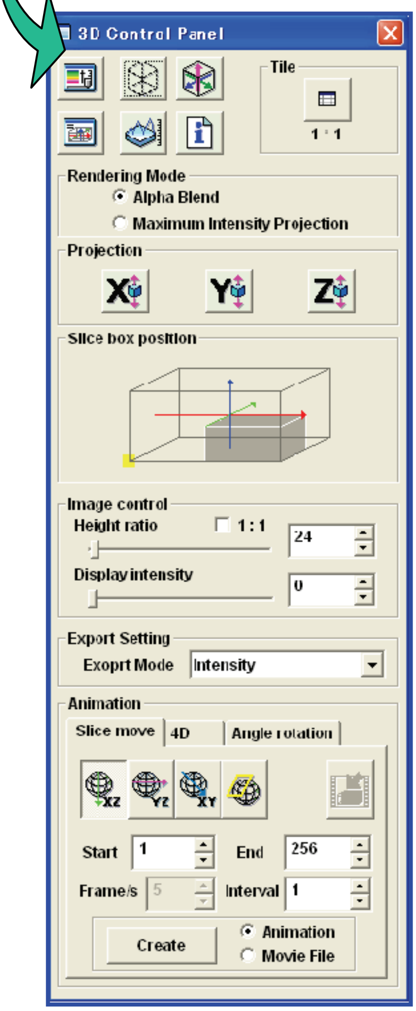
3D image rotation

Slice button

Change View Region button
Switches between the sliced 3D image view region and the hidden region.

Orthogonal View button
Allows for observation with the view point fixed at the front of a sliced cross-section.




3D Control Panel

Tile 1 : 1

Rendering Mode
● Alpha Blend
○ Maximum Intensity Projection

Projection X Y Z

Slice box position

Image control
Height ratio ☐ 1 : 1 24
Display intensity 0

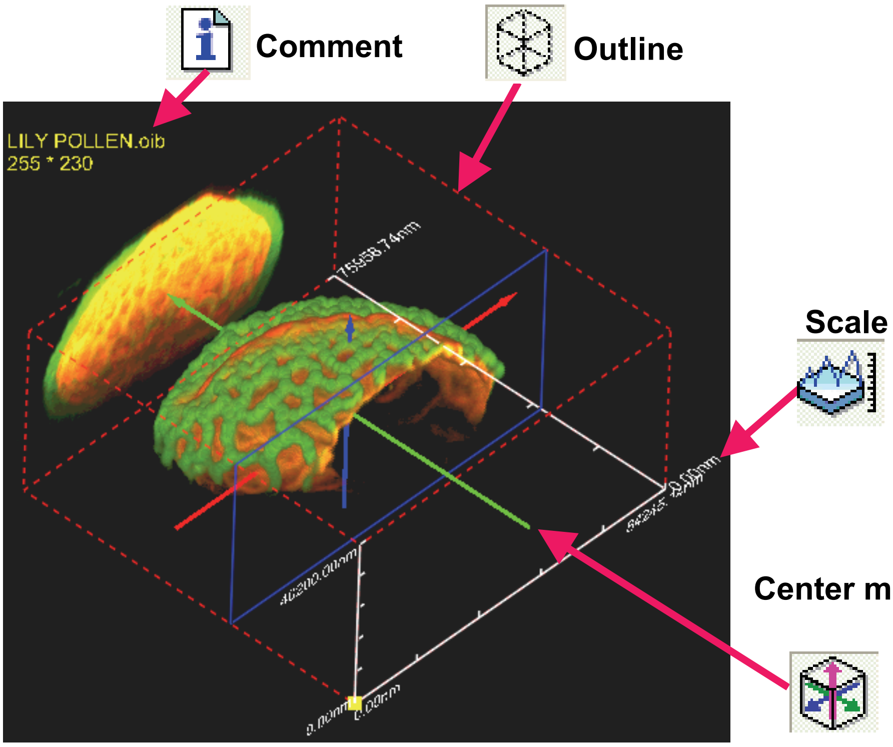
Export Setting
Exoprt Mode Intensity

Animation
Slice move | 4D | Angle rotation
Start 1 End 256
Frame/s 5 Interval 1
Create ● Animation ○ Movie File

3D Image Settings (LUT adjustment, image adjustment, etc.)

3D Property Settings (image graphics setting such as scale bars and comments)

Graphics ON/OFF



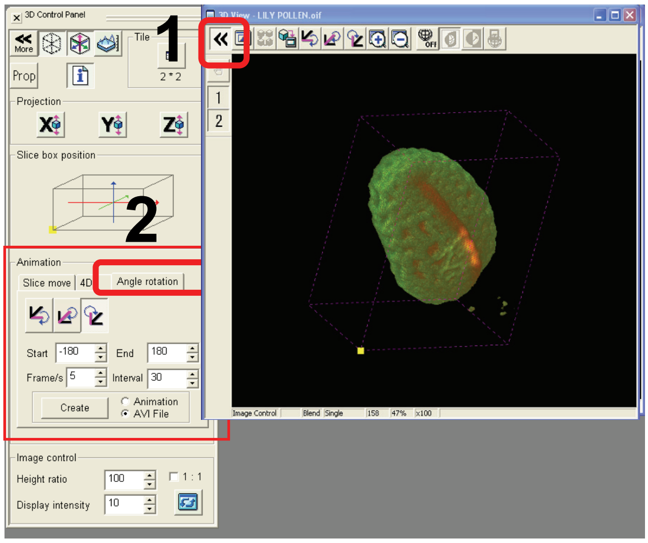
Comment

Outline

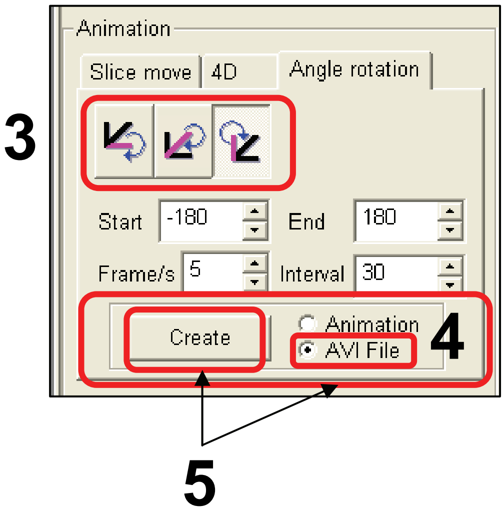
Scale

Center mark

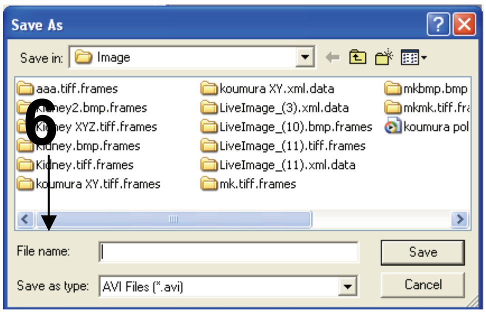
3D Image Analysis How to create a file


3D Control Panel
More
Tile
Prop
2*2
Projection
Slice box position
Animation
Slice move
4D
Angle rotation
Create
Animation
AVI File
Image control
Height ratio
Display intensity
1 : 1
3D View - LILY POLLEN.oif
Image Control
Blend
Single
1
2

Start -180 End 180
Frame/s 5 Interval 30
Height ratio 100
Display intensity 10


3
4
5
Animation
Slice move
4D
Angle rotation
Create
○ Animation
● AVI File

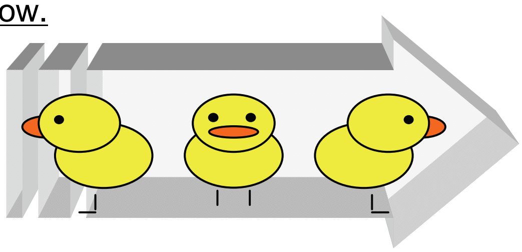
Start -180 End 180
Frame/s 5 Interval 30


6
Save As
aaa.tiff.frames
koumura XY.xml.data
mkbmp.bmp
LiveImage_(3).xml.data
LiveImage_(10).bmp.frames
LiveImage_(11).tiff.frames
LiveImage_(11).xml.data
mk.tiff.frames
Kidney.tiff.frames
Save
Cancel

Save in: Image
File name:
Save as type: AVI Files (*.avi)

To save a rotation file as a movie, render the image in 3D with the following procedure.

As an example, an image is rotated 180º below.



1. Click the « button.

2. Click the Angle rotation tab.

3. Select the rotation axis.

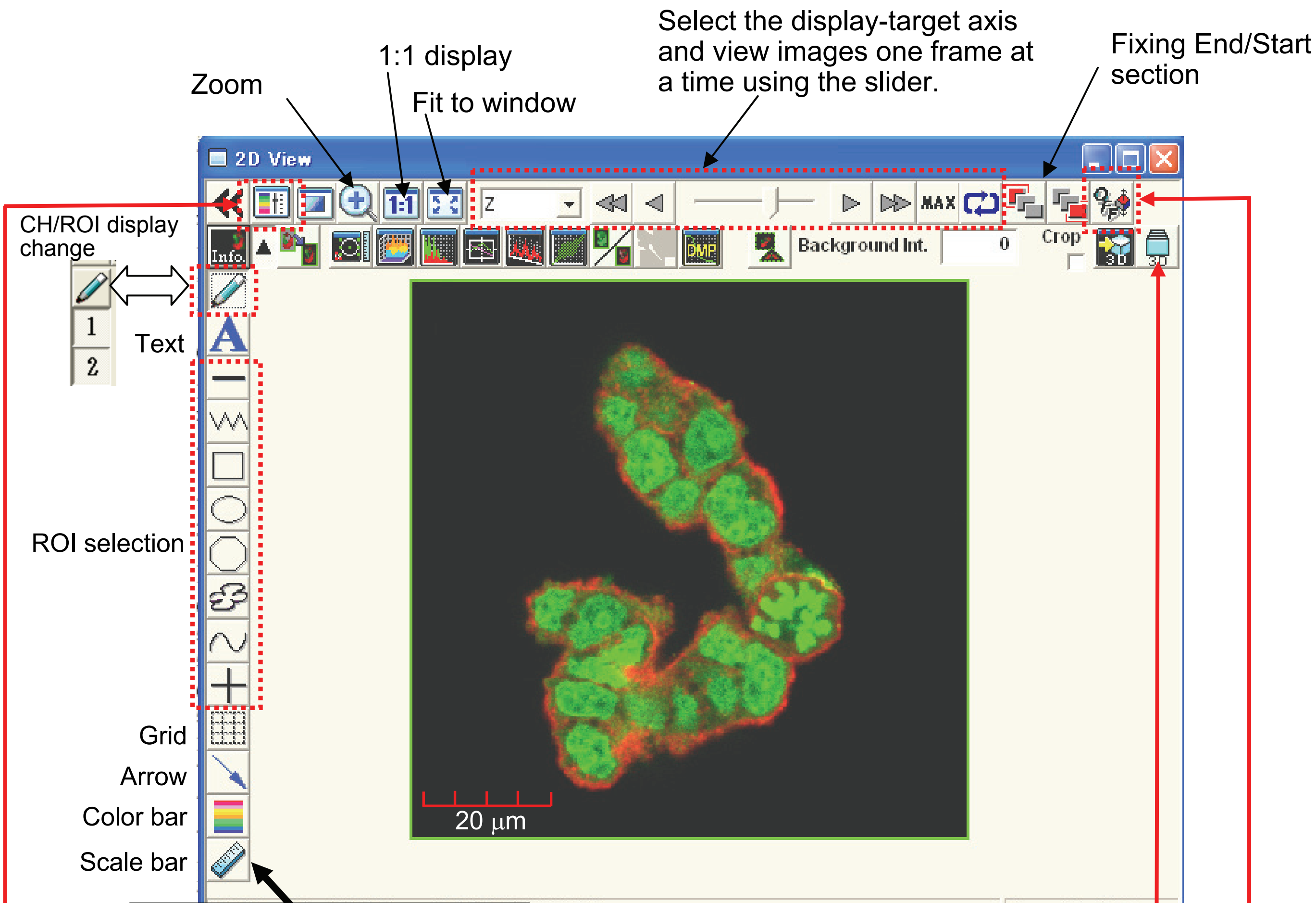
4. Enter the rotation angle.

Start: starting angle / End: ending angle
Frame/s: rotation speed / Interval: angle interval

5. Select AVI File and then click Create.

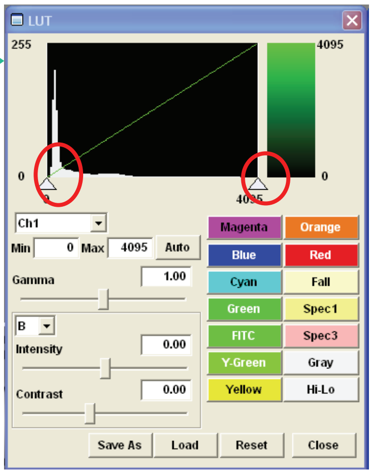
6. Enter a file name and then click Save.

2D View Window


Zoom
1:1 display
Fit to window
Select the display-target axis and view images one frame at a time using the slider.
Fixing End/Start section
CH/ROI display change
1
2
Text
ROI selection
Grid
Arrow
Color bar
Scale bar
2D View
Z
MAX
Info.
Background Int.
0
Crop
3D
20 µm
:144, 162
Zoom:Auto(74%)

Left-click and hold the button to drag it to a place on the image and drop it there.
(A straight line can be drawn by pressing Shift when left-clicking.)

LUT setting (edits image brightness)


LUT
255
4095
0
0
4095
Ch1
Min
0
Max
4095
Auto
Gamma
1.00
B
Intensity
0.00
Contrast
0.00
Magenta
Orange
Blue
Red
Cyan
Fall
Green
Spec1
FITC
Spec3
Y-Green
Gray
Yellow
Hi-Lo
Save As
Load
Reset
Close

Contrast can be freely set by moving the cursor right or left, or by entering values in Min and Max.

Creation of rotating 3D animation

Creates a rotating 3D animation based on the X axis (standard animation).

Creates a rotating 3D animation based on the Y axis (standard animation)

Displays the 3D Animation window that can be used to customize the settings of the rotating 3D animation creation process.
Enter settings and click New Image to create a 3D animation.

Projection mode change

Projection Off button: No projection displayed.

Z Intensity Projection button: Displays Z Intensity Projection images.

T Intensity Projection button: Displays T Intensity Projection images.

λ Intensity Projection button: Displays λ Intensity Projection images.

TOPO Z Topographic Projection button: Displays Z Topographic Projection images.

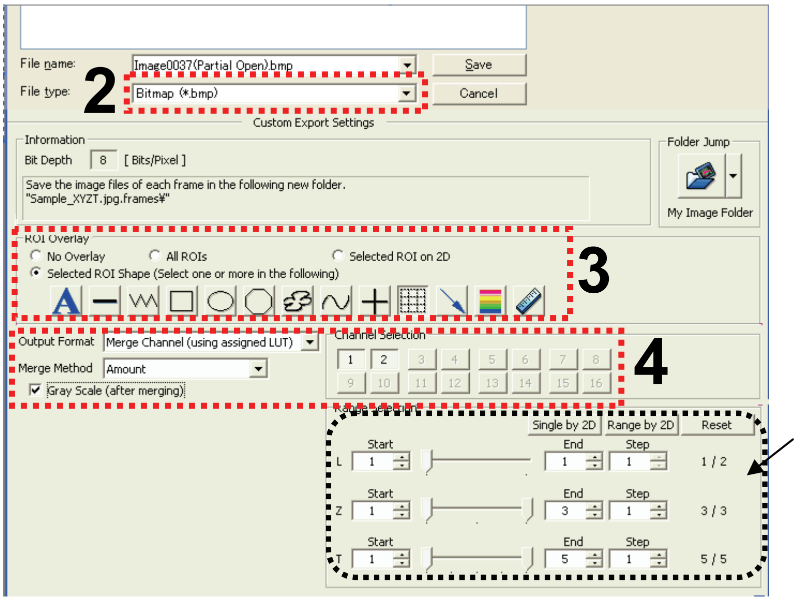
L TOPO λ Topographic Projection button: Displays λ Topographic Projection images.

T Series Average button: Displays T series average images.

Acquired Image Export

Saving images as Bitmap (*.bmp), JPEG (*.jpg, *.jpeg), TIFF (*.tif, *.tiff), or PNG (*.png)

1. Open the Live View window or 2D View window of the image to be saved. Right-click on the image and then select Export.
2. Select a file format to use for the export.
3. When saving a ROI in an image, such as a scale bar, select All ROIs or Selected ROI Shape.
4. Select the output mode of the image. In Channel Selection, select an output channel.
    - Merging images
    - LUT images for each CH
    - Raw data (black and white) images
5. To save the file, click Save.


2
3
4
Save
Cancel
Custom Export Settings
Information
Folder Jump
My Image Folder
ROI Overlay
Channel Selection
Range Selection
Single by 2D
Range by 2D
Reset

File name: Image0037(Partial Open).bmp

File type: Bitmap (*.bmp)

Bit Depth 8 [Bits/Pixel]

Save the image files of each frame in the following new folder.
"Sample_XYZT.jpg.frames¥"

○ No Overlay ○ All ROIs ○ Selected ROI on 2D

● Selected ROI Shape (Select one or more in the following)

Output Format: Merge Channel (using assigned LUT)

Merge Method: Amount

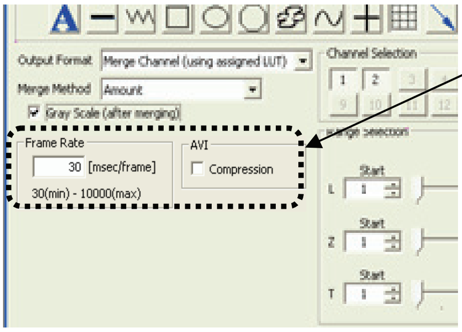
☑ Gray Scale (after merging)

	Start	End	Step	
L	1	1	1	1 / 2
Z	1	3	1	3 / 3
T	1	5	1	5 / 5

Range selection
Specify the Start and End values of axes to be read (L/Z/T).

Saving images in movie format (AVI or MOV) (The AVI or MOV format is available for XYZ, XYT, XYλ, XZT, XλT, and XλZ images.)

1. Open the Live View window or 2D View window of the image to be saved. Right-click on the image and then select Export.
2. Select a movie format and enter the frame rate in the Frame Rate field.
3. To save the file, click Save.


Channel Selection
Range Selection
Frame Rate
AVI

Output Format: Merge Channel (using assigned LUT)

Merge Method: Amount

☑ Gray Scale (after merging)

Frame Rate: 30 [msec/frame]
30(min) - 10000(max)

☐ Compression

Frame Rate
The frame rate of the movie and whether to set compression or not can be set.
(AVI: Sets whether to compress the AVI file.)

Only for SPD

Adjustment of Acquisition Wavelength Range (VBF)

The wavelength range of waves acquired in Ch1 and Ch2 can be adjusted by using the **Variable Barrier Filter**.

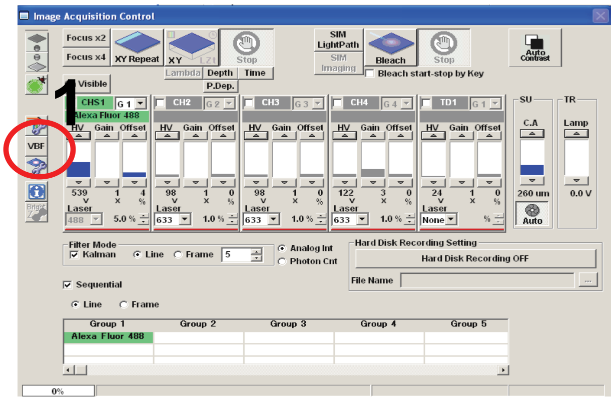
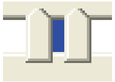
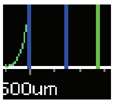
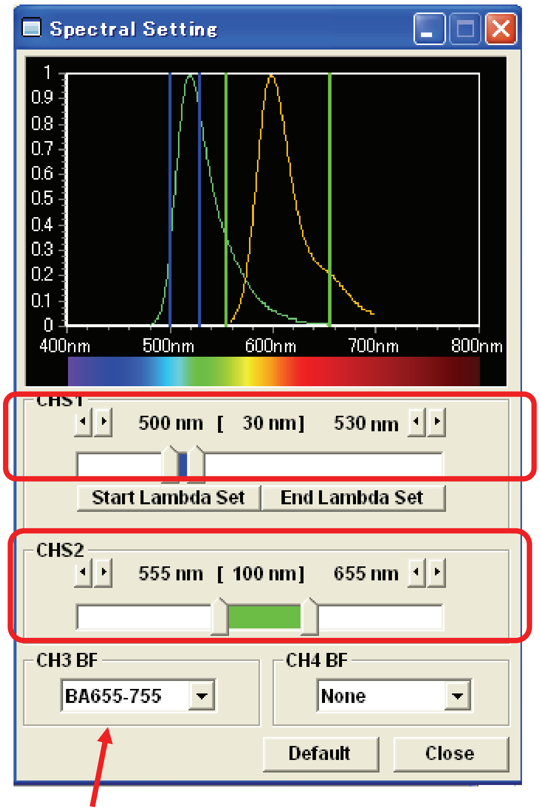


Image Acquisition Control
1
VBF

1. Click the VBF button to open the Spectral Setting window.

2. Using  or , move the position to adjust the wavelength of the wave to be acquired.

Spectral Setting window


Spectral Setting
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400nm
500nm
600nm
700nm
800nm

CHS1 500 nm [30 nm] 530 nm

Start Lambda Set | End Lambda Set

CHS2 555 nm [100 nm] 655 nm

CH3 BF BA655-755

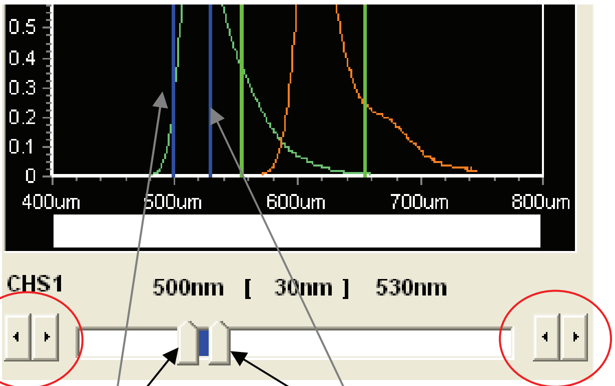
CH4 BF None

Default | Close

Slit adjustment for Ch1

Slit adjustment for Ch2

Fluorescent filter selection for Ch3


0.5
0.4
0.3
0.2
0.1
0
400um
500um
600um
700um
800um

CHS1 500nm [30nm] 530nm

Changes the **slit position**.

Changes the **slit width**.

Changes the **slit position** in 1 nm increments.

Changes the **slit width** in 1 nm increments.

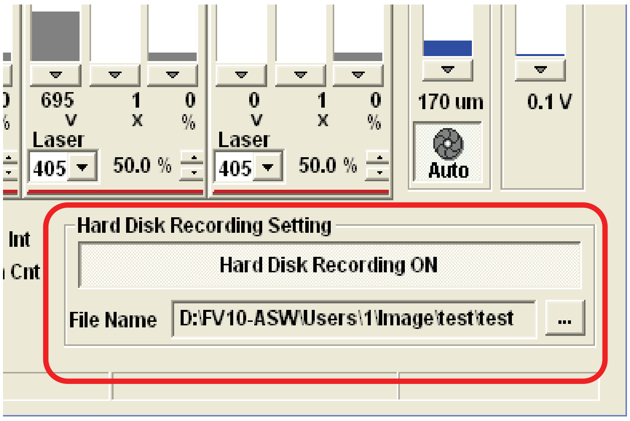
Hard Disk Recording

- This function automatically saves acquired images in a preset save destination.
- When series imaging is executed, you can check images up to the immediately preceding one through the Viewer window in the middle of the image acquisition process.

[Hard disk recording conditions]

Maximum number of image acquisition	32,766 times (number of images in series)
Save destination	Only local drives are guaranteed (D drive)
Other conditions	XYT images below 128×128 in size. Intervals of 1 second or more are required. Disabled for the reciprocating scan mode. **The review function is only available for interval operation.**

How to acquire images



Hard Disk Recording Setting

File Name D:\FV10-ASW\Users\1\Image\test\test

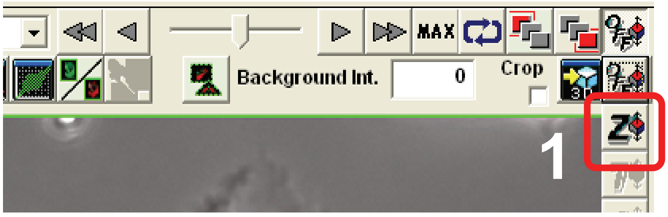
1. Set the image adjustment and condition settings.
2. Click the Hard Disk Recording OFF button. The button appears as Hard Disk Recording On and the Viewer window opens.
3. Click the ... button next to the File Name textbox. Set the save destination and file name and then click Open.
4. To acquire images, click the XY LZt button.

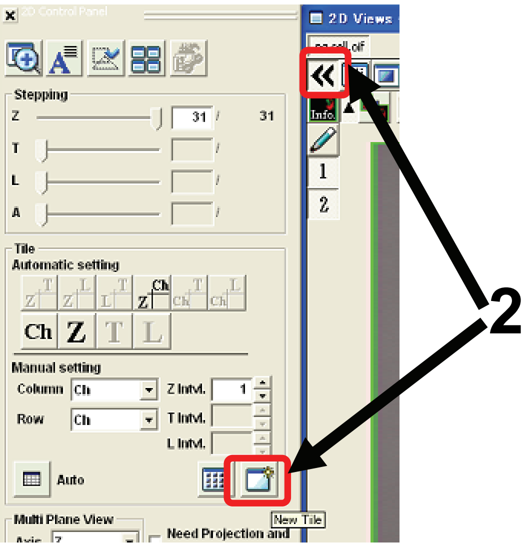
Viewing images in the middle of the image acquisition process

1. After image acquisition starts, bring the Viewer window to the front.
2. Double-click an image file during the acquisition process with the view set to Thumbnail in the Explorer window.

When image files are not displayed in the Thumbnail view during the acquisition process, right-click and select Refresh.

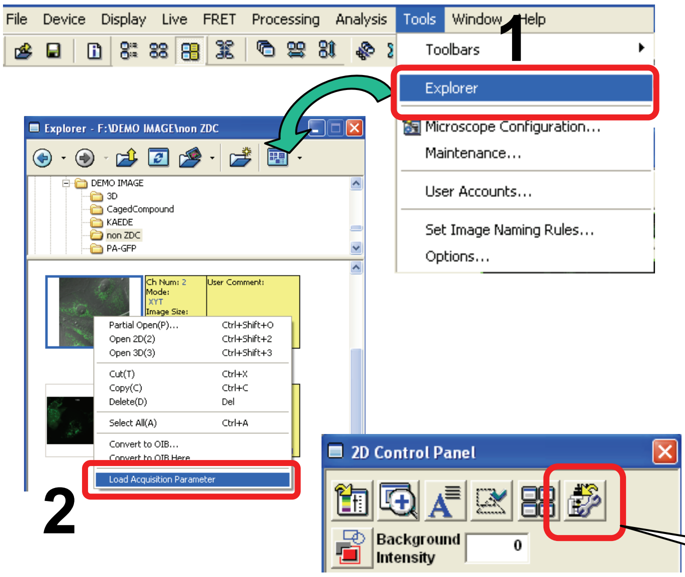
A. Creation of 2D Z Projection Images
B. Reloading Set Conditions


MAX
Background Int.
0
Crop
1


2D Control Panel
2D Views
Stepping
Z
31
31
T
L
A
Tile
Automatic setting
Ch
Z
T
L
Manual setting
Column
Ch
Z Intvl.
1
Row
Ch
T Intvl.
L Intvl.
Auto
New Tile
Multi Plane View
Need Projection and
Info.
1
2
2

A. Creation of 2D Z projection images

1. Open an original image and then select Z from the Projection button in the 2D View to open the Z projection image.
2. Click the New Tile button in the 2D Control Panel (opened by clicking «) to create a 2D image of the Z projection image.
3. Save the image.


File
Device
Display
Live
FRET
Processing
Analysis
Tools
Window
Help
Toolbars
Explorer
Microscope Configuration…
Maintenance…
User Accounts…
Set Image Naming Rules…
Options…
1
Explorer - F:\DEMO IMAGE\non ZDC
DEMO IMAGE
3D
CagedCompound
KAEDE
non ZDC
PA-GFP
Ch Num: 2
Mode: XYT
Image Size:
User Comment:
Partial Open(P)… Ctrl+Shift+O
Open 2D(2) Ctrl+Shift+2
Open 3D(3) Ctrl+Shift+3
Cut(T) Ctrl+X
Copy(C) Ctrl+C
Delete(D) Del
Select All(A) Ctrl+A
Convert to OIB…
Convert to OIB Here
Load Acquisition Parameter
2
2D Control Panel
Background Intensity
0

B. Reloading set conditions

Loads image acquisition conditions from past image data in order to duplicate them.

1. Select Explorer from Tools.
2. Right-click on a thumbnail image from which to reload set conditions, and then select Load Acquisition Parameter.
3. The conditions are reloaded.

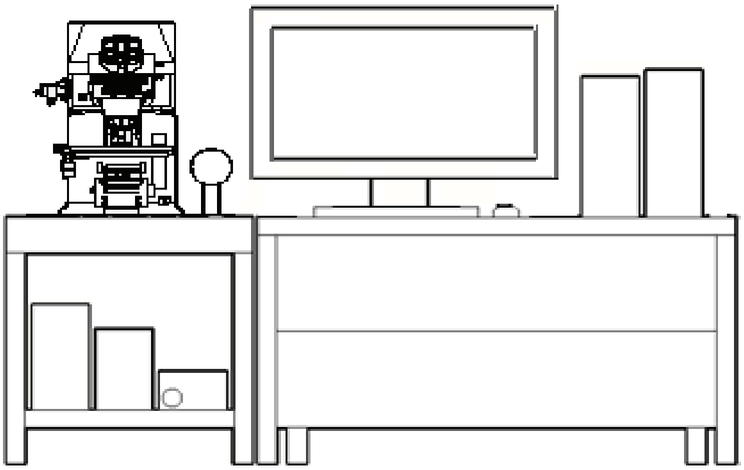
Conditions can be reloaded by clicking the Load Scan Data button while the image from which to reload conditions is open.

Laser Scanning Biological Microscope

FV1200

BX61

Quick Start



FV1200 BX61
Quick Start
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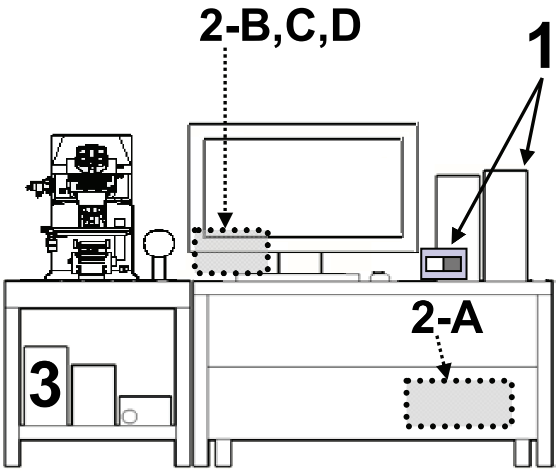
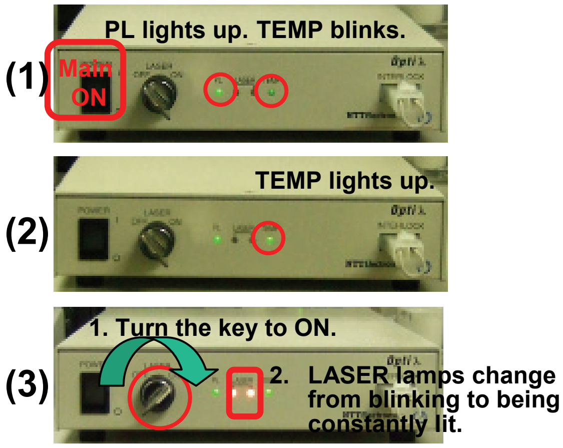
Image Acquisition

— Fundamentals —

System Startup



B: LD559 laser



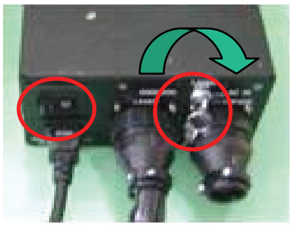
PL lights up. TEMP blinks.

TEMP lights up.

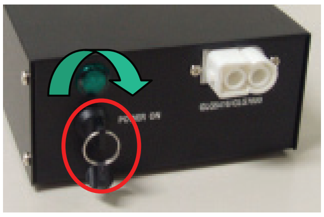
1. Turn the key to ON.

2. LASER lamps change from blinking to being constantly lit.

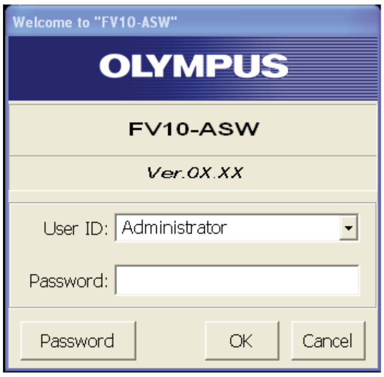
C: MAr laser



D: HeNe (G) laser



5



Welcome to "FV10-ASW"

OLYMPUS

FV10-ASW

Ver.0X.XX

User ID: Administrator

Password:

1. Switch on the central power and then start up the Controller.

2. Start up the laser systems.

A: MCPSU (405/440/473/559/635 nm)

Switch on the system.

B: LD559

(1) Switch on the main power of the laser system. The PL lamp lights up in **green** and the TEMP lamp blinks in **green**.

(2) Confirm that the TEMP lamp has changed from blinking to being constantly lit.

(3) Turn on the key switch. The LASER lamps **blink in red**. It takes a while for the lasers to stabilize (The lamps change from **blinking in red** to being constantly **lit in red**). After the LASER lamps are **lit in red**, the LD559 laser system is ready for use.

C: MultiAr laser

Switch on the main power and then turn the key switch clockwise.

D: HeNe (G) laser (543 nm)

Turn the key switch clockwise.

3. Switch on the power of the mercury lamp unit. *** Used for visual observation**

4. Enter the user name and password to log on to Windows.

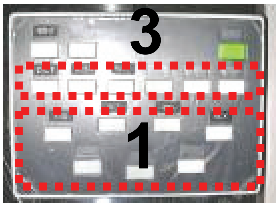
OS	User name	Passwprd
Windows7 (64bit)	**olympus**	**olympus**
Others	**fluoview**	**fluoview**

5. Double click FV to start up the FV10-ASW software.

User name: Administrator
Password: Administrator

Visual Observation via Microscope

■ ■ Fluorescent image observation ■ ■



Hand switches

■ Memo ■
Fluorescent mirror unit

WBV:	**blue violet excitation (ECFP, etc.)**
NIBA:	**blue excitation/green fluorescence (Example: FITC, EGFP, etc.)**
WIG:	**green excitation/red fluorescence (Example: rhodamine, DsRed, etc.)**
WU:	**UV excitation**

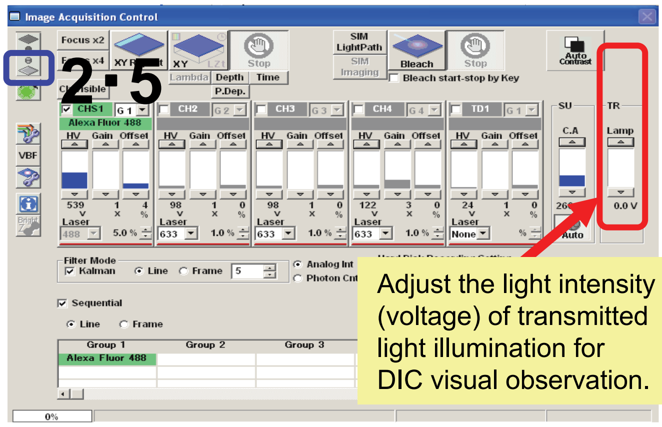
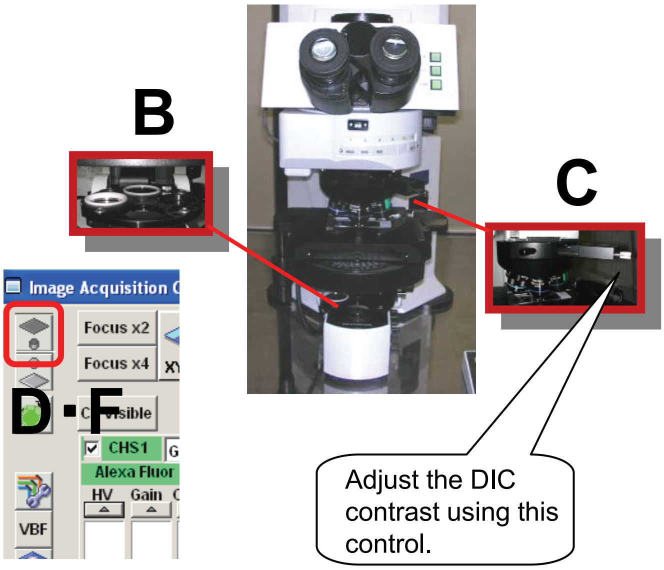


Image Acquisition Control
2·5
Focus x2
Focus x4
XY
Lambda
Depth
Time
Stop
SIM LightPath
SIM Imaging
Bleach
Bleach start-stop by Key
Auto Contrast
CHS1
CH2
CH3
CH4
TD1
Alexa Fluor 488
HV
Gain
Offset
Laser
SU
TR
C.A
Lamp
0.0 V
Auto
VBF
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Sequential
Group 1
Group 2
Group 3

Adjust the light intensity (voltage) of transmitted light illumination for DIC visual observation.

1. Select an objective lens using the hand switches.
2. Click in the FV10-ASW software.
3. Select a fluorescent mirror unit using the hand switches.
4. Adjust the focus.
5. After observation finishes, click to close the shutter of the fluorescent lamp.

■ ■ DIC image observation ■ ■


B
C
D·F
Image Acquisition C
Focus x2
Focus x4
CHS1
Alexa Fluor
HV
Gain
VBF

Adjust the DIC contrast using this control.

A. Select an objective lens using the hand switches.

B. Insert the polarizer.

C. Insert the DIC slider.
* Switch the prism position change lever to BFP1 or NORMAL depending on the objective lens used.

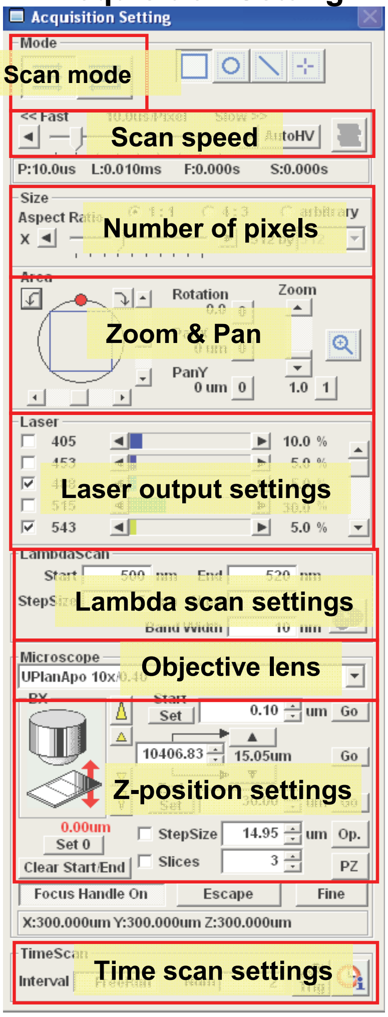
D. Click in the FV10-ASW software.

E. Adjust the focus.

F. After observation finishes, click to close the shutter of the halogen lamp.

Overview of Image Acquisition Panel

■ Acquisition Setting ■



Scan mode

Scan speed

Number of pixels

Zoom & Pan

Laser output settings

Lambda scan settings

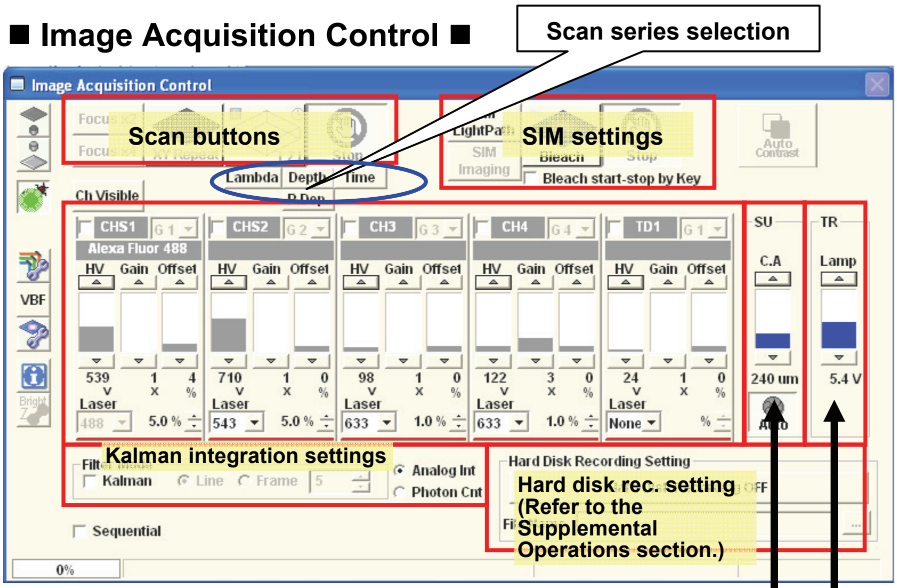
Objective lens

Z-position settings

Time scan settings

Acquisition Setting
Mode
<< Fast
AutoHV
P:10.0us L:0.010ms F:0.000s S:0.000s
Size
Aspect Ratio
Area
Rotation
Zoom
PanY
Laser
405
10.0 %
543
5.0 %
LambdaScan
Microscope
UPlanApo 10x
Set
Go
10406.83
15.05um
0.00um
Set 0
StepSize
14.95
Slices
3
Clear Start/End
Op.
PZ
Focus Handle On
Escape
Fine
X:300.000um Y:300.000um Z:300.000um
TimeScan
Interval

■ Image Acquisition Control ■



Scan series selection

Scan buttons

SIM settings

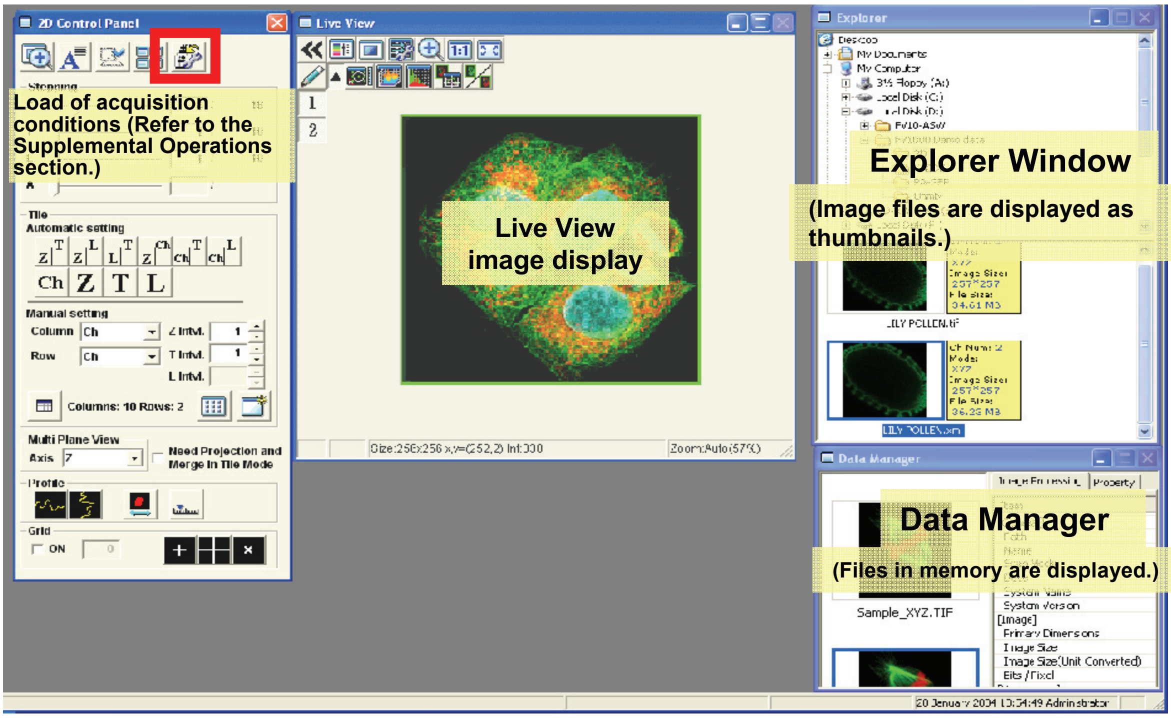
Kalman integration settings

Hard disk rec. setting (Refer to the Supplemental Operations section.)

Confocal aperture (pinhole diameter)

Transmitted-light lamp intensity control

Image Acquisition Control
Lambda
Depth
Time
Bleach start-stop by Key
Auto Contrast
Ch Visible
CHS1
CHS2
CH3
CH4
TD1
Alexa Fluor 488
HV
Gain
Offset
Laser
539
710
98
122
24
488
543
633
None
5.0 %
1.0 %
SU
C.A
240 um
TR
Lamp
5.4 V
VBF
Kalman
Line
Frame
Analog Int
Photon Cnt
Hard Disk Recording Setting
Sequential
0%



Load of acquisition conditions (Refer to the Supplemental Operations section.)

Live View image display

Explorer Window

(Image files are displayed as thumbnails.)

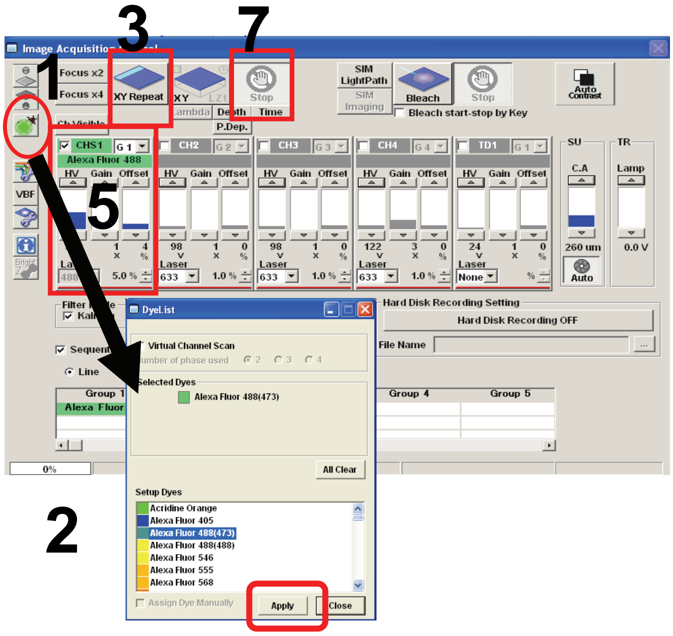
Data Manager

(Files in memory are displayed.)

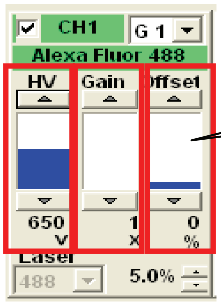
2D Control Panel
Live View
Explorer
Data Manager
Tile
Automatic setting
Manual setting
Column
Row
Columns: 10 Rows: 2
Multi Plane View
Axis
Profile
Grid
ON
Sample_XYZ.TIF

XY Image Acquisition (Single-color staining)

Example: single-color staining with green fluorescence (Alexa488)


1
3
7
5
2
Image Acquisition
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
P.Dep.
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
CH1
G 1
Alexa Fluor 488
CH2
CH3
CH4
TD1
HV
Gain
Offset
Laser
SU
C.A
260 um
Auto
TR
Lamp
0.0 V
Hard Disk Recording Setting
Hard Disk Recording OFF
File Name
Group 1
Group 4
Group 5
Sequential
Line
Dye.list
Virtual Channel Scan
Number of phase used
Selected Dyes
Alexa Fluor 488(473)
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close

5


CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650
V
1
X
0
%
Laser
488
5.0%

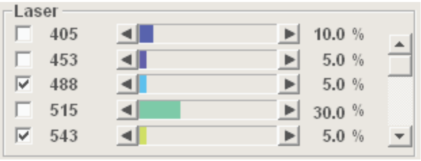
Ch group box adjustment

HV: Adjusts detector sensitivity.

Gain: Brightens images with a set magnification.

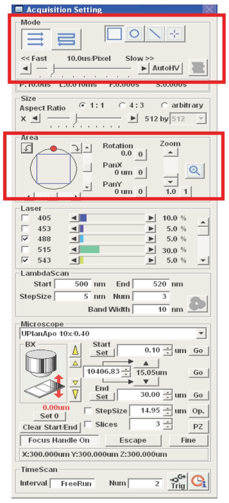
Offset: Darkens the background.

Laser intensity adjustment



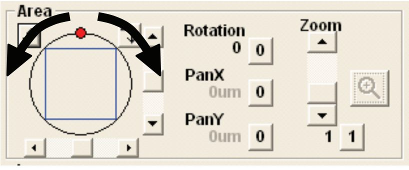
Laser

☐	405	10.0 %
☐	453	5.0 %
☑	488	5.0 %
☐	515	30.0 %
☑	543	5.0 %

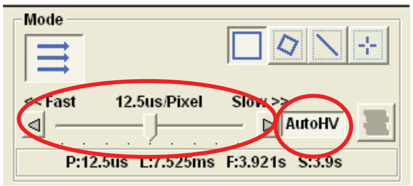

Acquisition Setting
Mode
Fast
Slow
AutoHV
Size
Aspect Ratio
Area
Rotation
Zoom
PanX
PanY
Laser
LambdaScan
Microscope
UPlanApo 10x 0.40
TimeScan

6

Rotation **Zoom**


Area
Rotation
0
Zoom
PanX
0um
0
PanY
0um
0
1
1

8 **Rotation**


Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us L:7.525ms F:3.921s S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
The slit width and positions can be changed manually.
For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Click the XY Repeat button XY Repeat to execute a continuous scan.

* If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4.

4. Adjust the focus by turning the focus knob.

5. Make adjustments for green imaging (Alexa488) (laser intensity, HV, offset, etc.).

6. Set the zoom and rotation as required.

7. After finishing image adjustment, click the STOP button Stop to stop scanning.

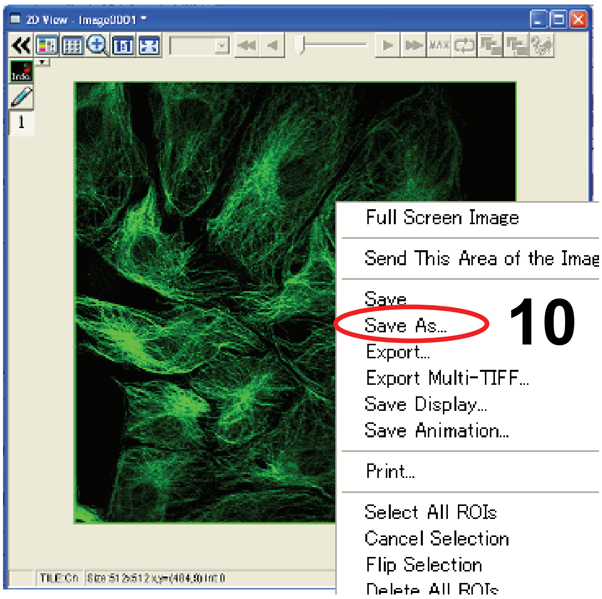
8. Select AutoHV and then select ScanSpeed.

* The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
Alternatively, Kalman integration can be used.
For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Single-color staining)

Example: single-color staining with green fluorescence (Alexa488)

9


2D View - Image0001
Full Screen Image
Send This Area of the Image
Save
Save As...
10
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

9. To acquire the image, click the XY button.
10. Save the image: Select **Save As** by right-clicking or going to the File menu, and then save the image in OIB or OIF format.

> **■ Memo ■**
> **FV10-ASW dedicated file formats**
>
> OIF format:
> Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.
>
> OIB format:
> In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an image as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

[Points on acquiring clear images]

For dark images

Increasing the HV: Increases detector sensitivity. Use another method in addition to this if sufficient brightness cannot be obtained even when the HV is increased to 700 V or higher.

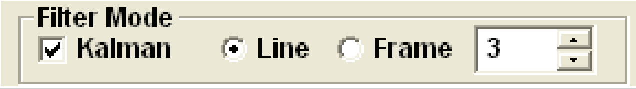
Increasing laser intensity: Increases fluorescent brightness with an increase in excitation light output. Note that an excessively increased intensity may fade fluorescence or cause greater damage to samples.

Increasing the CA: The Auto button stays clicked during regular use so that the optimal pinhole diameter is always automatically set.
Higher brightness can be obtained by deselecting Auto to increase the CA (confocal aperture), which is equivalent to decreasing the Z-axis resolution.

Increasing the gain: Brightens images with the magnification set before image acquisition.

For noisy images

Kalman integration ... Averages images while acquiring as many images as specified. Check the checkbox for Kalman and then select Line (or Frame). Enter **the number of image integrations (the number of scans)**.

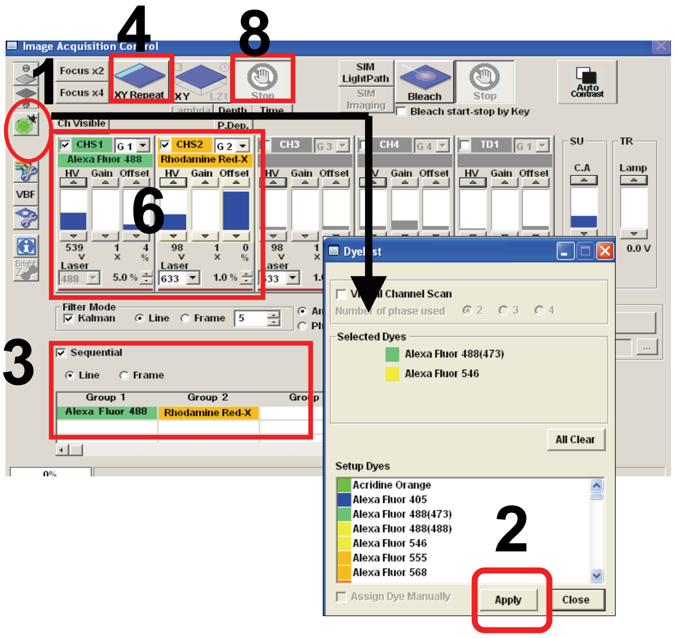


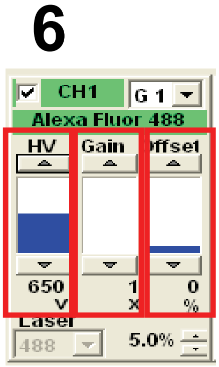
Filter Mode
☑ Kalman ● Line ○ Frame 3

XY Image Acquisition (Multicolor staining)

Line sequential scan

* Mode for avoiding fluorescent crosstalk by individually acquiring fluorescent images


4
8
1
6
3
2
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
Ch Visible
P.Dep.
Lambda
Depth
Time
CH1
G 1
Alexa Fluor 488
CH2
G 2
Rhodamine Red-X
CH3
CH4
TD1
SU
TR
C.A
Lamp
HV
Gain
Offset
Laser
Filter Mode
Kalman
Line
Frame
Sequential
Line
Frame
Group 1
Group 2
Alexa Fluor 488
Rhodamine Red-X
DyeList
Virtual Channel Scan
Number of phase used
Selected Dyes
Alexa Fluor 488(473)
Alexa Fluor 546
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close


6
CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650
V
1
X
0
%
Laser
488
5.0%

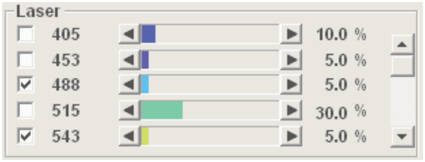
Ch group box adjustment

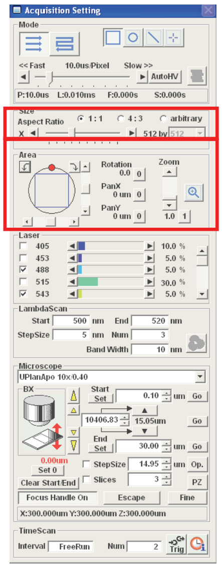
HV: Adjusts detector sensitivity.

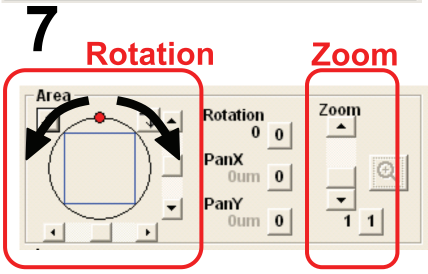
Gain: Brightens images with a set magnification.

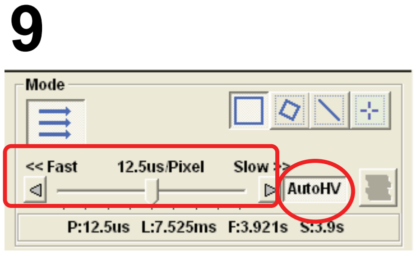
Offset: Darkens the background.

Laser intensity adjustment


Laser
405
10.0 %
453
5.0 %
488
5.0 %
515
30.0 %
543
5.0 %


Acquisition Setting
Mode
<< Fast
10.0us/Pixel
Slow >>
AutoHV
Size
Aspect Ratio
1 : 1
4 : 3
arbitrary
512 by 512
Area
Rotation
Zoom
PanX
PanY
Laser
LambdaScan
Microscope
UPlanApo 10x 0.40
TimeScan


7
Rotation
Zoom
Area
Rotation
0
PanX
0um
PanY
0um
Zoom
1


9
Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us
L:7.525ms
F:3.921s
S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Check the checkbox for Sequential and then select Line.

4. Click the XY Repeat button XY Repeat to execute a continuous scan.

   * If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4 .

5. Adjust the focus by turning the focus knob.

6. Make adjustments for CH1/CH2 imaging (laser intensity, HV, offset, etc.).

7. Set the zoom and rotation as required.

8. After finishing image adjustment, click the STOP button Stop to stop scanning.

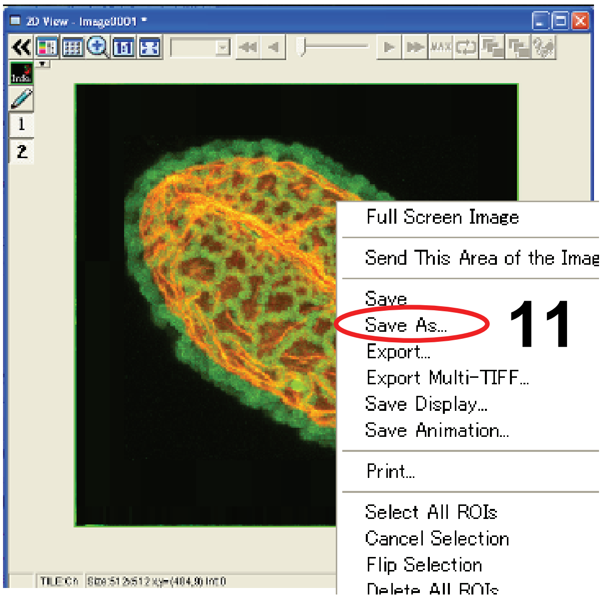
9. Select AutoHV and then select ScanSpeed.

   * The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
   Alternatively, Kalman integration can be used.
   For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Multicolor staining)

Line sequential scan

10


2D View - Image0001
1
2
Full Screen Image
Send This Area of the Imag
Save
Save As...
11
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

10. To acquire the image, click the XY button.
11. Save the image: Select **Save As** by right-clicking or going to the File menu, and then save the image in OIB or OIF format.

■ Memo ■
FV10-ASW dedicated file formats

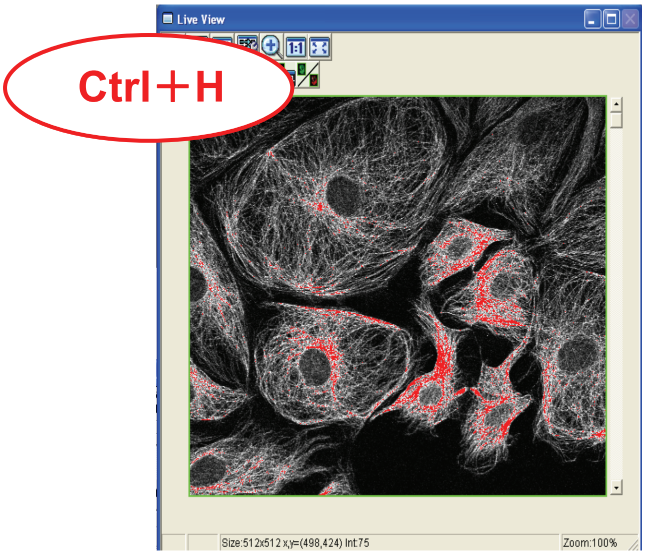
OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

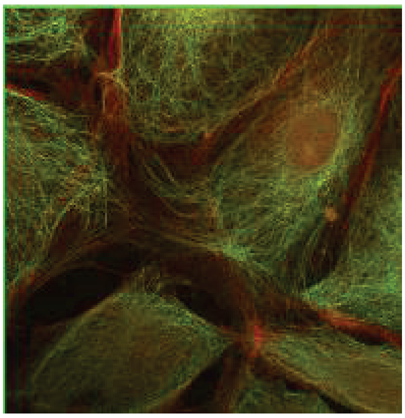
OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an image as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

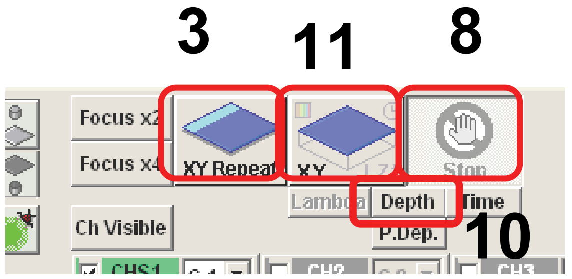
■ Image brightness adjustment ■

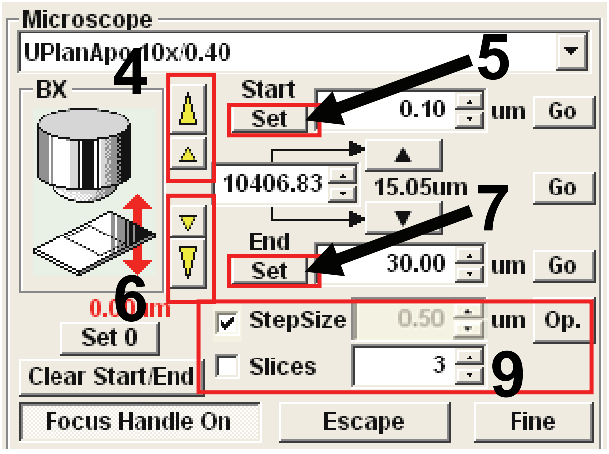
By pressing **Ctrl + H** on the keyboard, an image can be displayed in Hi-Low mode. Pixels of intensity 4095 are shown in red.
To return to normal mode from Hi-Low mode, press **Ctrl + H** again. The image returns to its original color.


Live View
Ctrl+H
Size:512x512 x,y=(498,424) Int:75
Zoom:100%



XYZ Image Acquisition

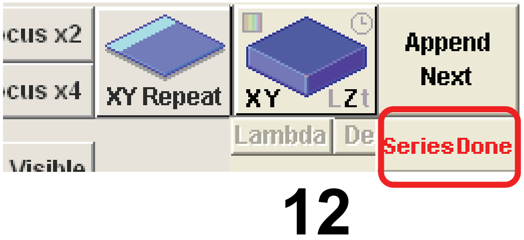

3
11
8
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
P.Dep.
10
Ch Visible
CHS1
CH2
CH3


Microscope
UPlanApo 10x/0.40
BX
4
5
Start
Set
0.10
um
Go
10406.83
15.05um
Go
7
End
Set
30.00
um
Go
6
0.00um
Set 0
Clear Start/End
StepSize
0.50
um
Op.
Slices
3
9
Focus Handle On
Escape
Fine

■ Memo ■

▲ : Each click shifts the position by the value entered for StepSize.

▵ : Each click shifts the position by 1/2 the value entered for StepSize.


Focus x2
Focus x4
XY Repeat
XY
LZt
Append
Next
Lambda
De
Series Done
Visible
12

1. Before starting the following procedure, make adjustments for XY imaging.
   (Refer to page 6 or 8.)

The following procedure fixes the upper and lower limit positions of a continuous cross-sectional image.

2. Click Clear Start/End to clear the settings of acquisition start and end position. Then, click Set 0 to zero the Z-position values being displayed.

3. Click the XY Repeat button to execute a scan.

4. Click ▲ or ▵ to shift the focus.
   (Refer to ■ Memo 1 ■.)

5. When the upper limit position of the sample is displayed in the image, fix the position by clicking the Set button.

6. Click ▼ or ▿ to shift the focus.
   (Refer to ■ Memo 1 ■.)

7. When the lower limit position of the sample is displayed in the image, fix the position by clicking the Set button.

8. To stop scanning, click the Stop button.

9. Enter a value in StepSize or Slices, and check ☑ the checkbox.
   (Click the Op button to see a recommended value.)

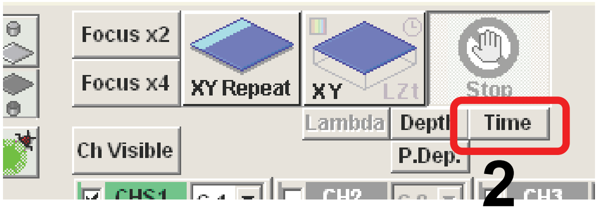
10. Select Depth.

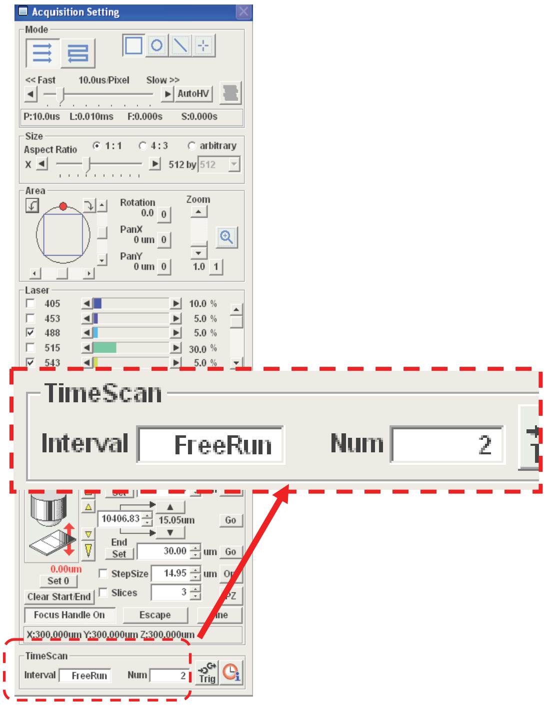
11. To acquire the image, click the XYZ button.

12. To save the image, click Series Done.

For more information on 3D image display, refer to the Supplemental Operations section.

XYT Image Acquisition

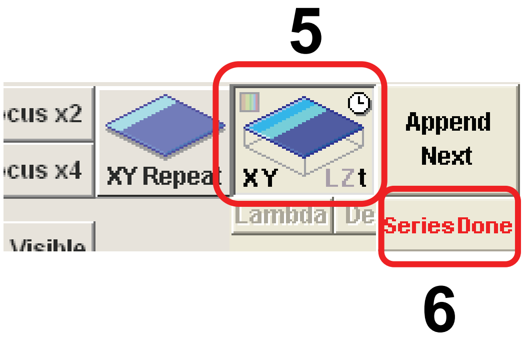

Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
P.Dep.
Ch Visible
CHS1
CH2
CH3
2


Acquisition Setting
Mode
Size
Area
Laser
TimeScan

TimeScan

Interval FreeRun

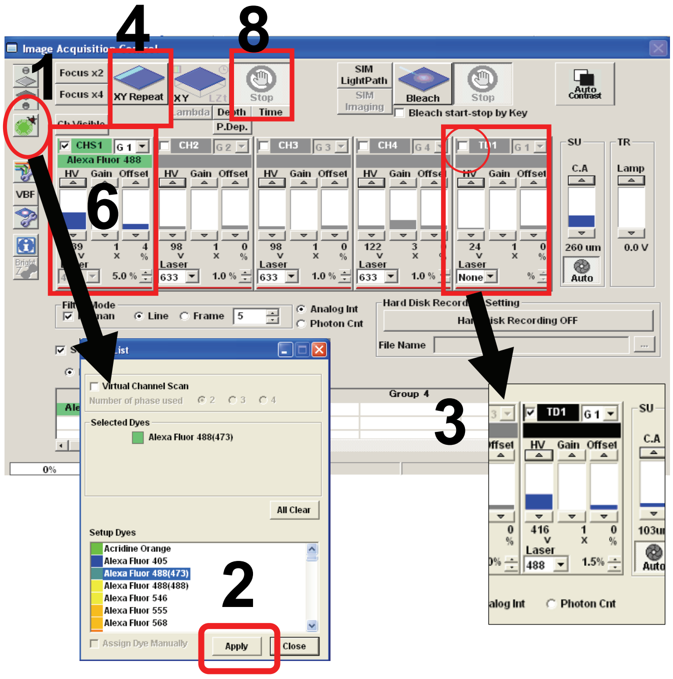
Num 2

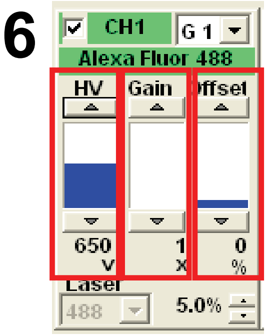

5
cus x2
cus x4
XY Repeat
XY
LZt
Append
Next
Lambda
De
Series Done
Visible
6

1. Before starting the following procedure, make adjustments for XY imaging.
   (Refer to page 6 or 8.)
2. Select Time from among the series buttons.
3. In the Interval box, enter the time (in seconds) taken between one acquisition start and the next acquisition start.
   Example: To set a 1 minute interval, type “60” and click Enter.
4. Enter the number of acquisition in the Num box.
5. To acquire the images, click the XYT button.
6. To save the images, click Series Done.

XY Image Acquisition (Single-color staining + DIC)

Example: green fluorescent + DIC image

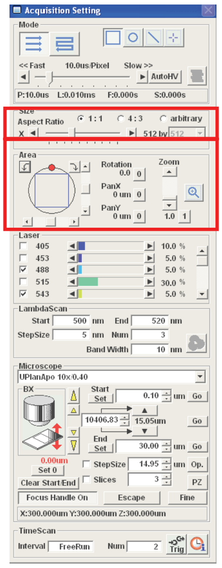

4
8
1
6
3
2
Apply

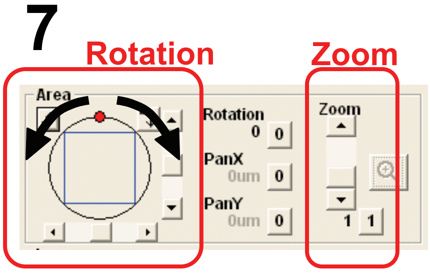

6
CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650 V
1 X
0 %
Laser
488
5.0%

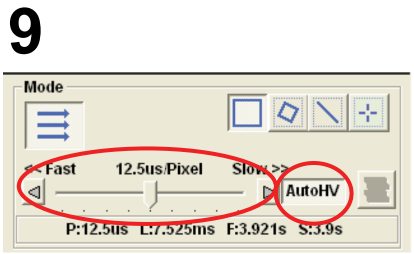
HV: Adjusts detector sensitivity.

Gain: Brightens images with a set magnification.

Offset: Darkens the background.


Acquisition Setting


7
Rotation
Zoom
Area
Rotation 0
PanX 0um 0
PanY 0um 0
Zoom 1


9
Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us L:7.525ms F:3.921s S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

   Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
   The slit width and positions can be changed manually.
   For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Check the checkbox for TD1.

   Check that the polarizer and the DIC slider for transmitted light are within the optical path limits.

4. Click the XY Repeat button XY Repeat to execute a continuous scan.

   * If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4.

5. Adjust the focus by turning the focus knob.

6. Make adjustments for green imaging (Alexa488) and TD imaging.

7. Set the zoom and rotation as required.

8. After finishing image adjustment, click the STOP button Stop to stop scanning.

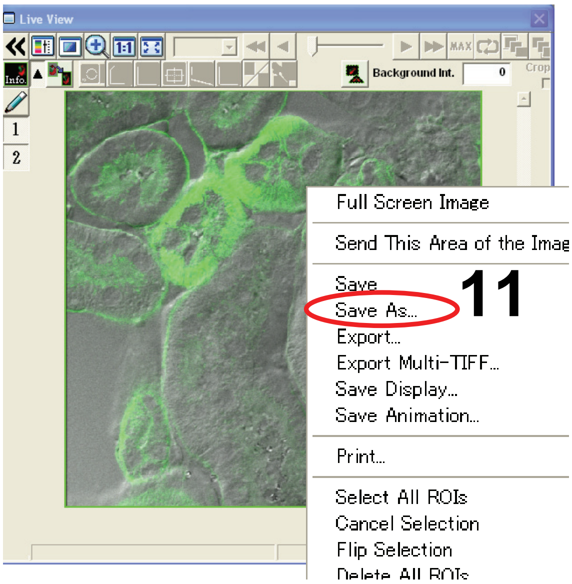
9. Select AutoHV and then select ScanSpeed.

   * The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
   Alternatively, Kalman integration can be used.
   For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Single-color staining + DIC)

Example: green fluorescent + DIC image

10


Live View
Info
Background Int.
0
MAX
Crop
1
2
Full Screen Image
Send This Area of the Imag
Save
Save As...
11
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

10. To acquire the image, click the XY button.

11. Save the image: Select **Save As** by right-clicking or going to the File menu and then save the image in OIB or OIF format.

■ Memo ■
FV10-ASW dedicated file formats

OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

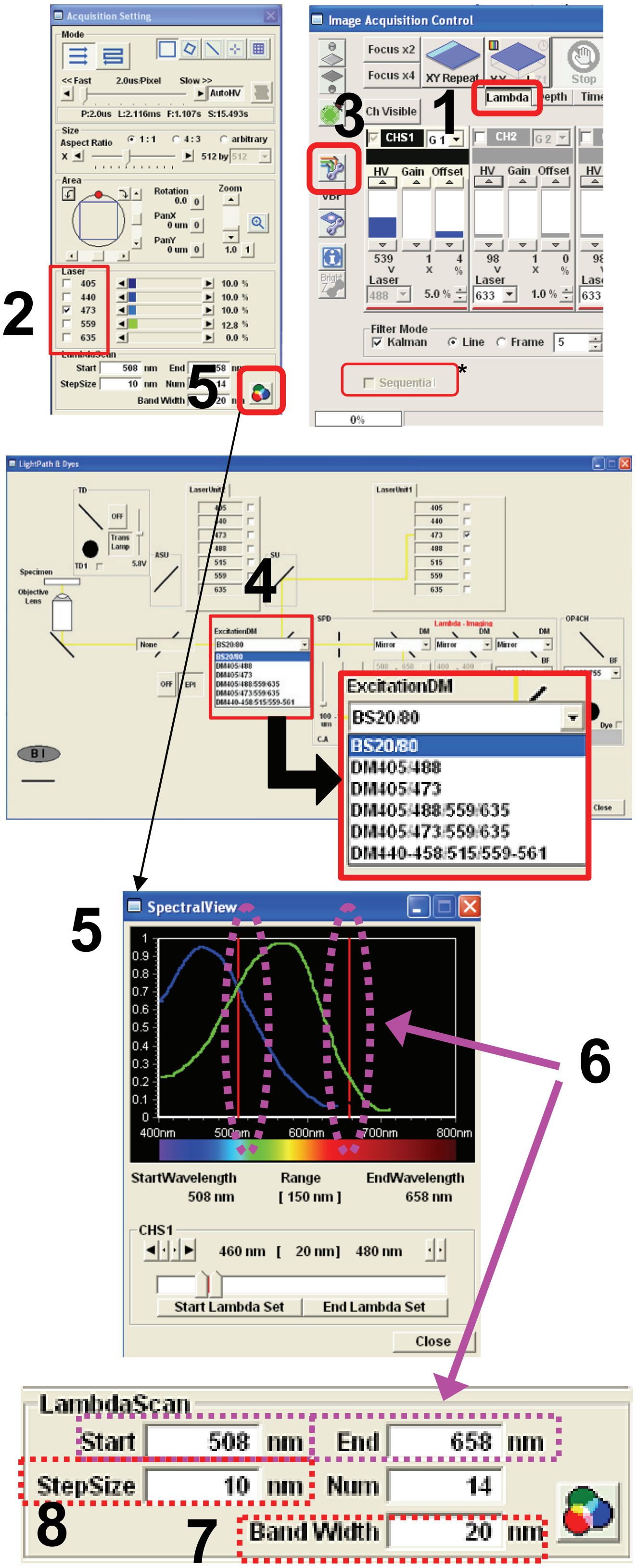
OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an images as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

Only for SPD

XYλ Image Acquisition

Spectral image acquisition



Acquisition Setting
Image Acquisition Control
Lambda
Sequential
LightPath & Dyes
ExcitationDM
SpectralView
1
2
3
4
5
5
6
7
8
*

ExcitationDM: BS20/80

- BS20/80
- DM405/488
- DM405/473
- DM405/488/559/635
- DM405/473/559/635
- DM440-458/515/559-561

SpectralView: StartWavelength 508 nm, Range [150 nm], EndWavelength 658 nm

CHS1: 460 nm [20 nm] 480 nm

LambdaScan: Start 508 nm, End 658 nm, StepSize 10 nm, Num 14, Band Width 20 nm

1. Click **Lambda** .

   * When the Sequential checkbox is checked, the Lambda button is grayed out. Uncheck the checkbox.

2. Check the checkbox for the laser to be used.

3. To display the optical path diagram, click .

4. Select BS20/80 or a DM according to the excitation wavelength.

   Example: Select DM405/473 for excitation at 473.

5. To display SpectralView, click .

6. In the Start and End boxes, enter the wavelength at which to start and end image acquisition.

   These boxes correspond to the red bars in the SpectralView graph.

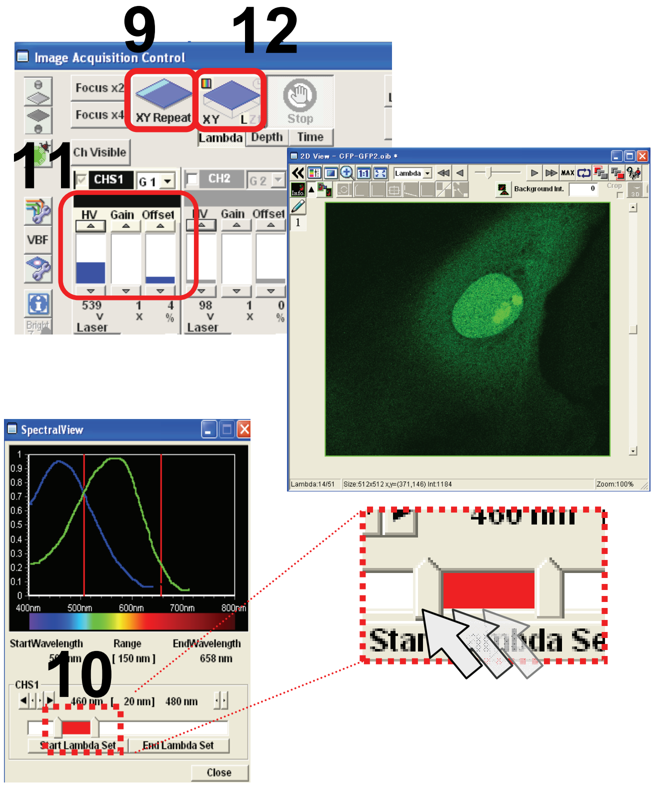
7. In the Band Width box, set the wavelength range of acquisition per frame (Example: 20 nm).

8. In the StepSize box, enter the wavelength interval between one acquisition start and the next acquisition start (Example: 10 nm).

   The minimum settable unit is 1.0 nm.

XYλ Image Acquisition

Spectral image acquisition


9
12
11
10
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
Ch Visible
CHS1
CH2
HV
Gain
Offset
VBF
Laser
2D View - GFP-GFP2.oib
SpectralView
StartWavelength
Range
EndWavelength
CHS1
Start Lambda Set
End Lambda Set
Close

9. To execute a scan, click the XY Repeat button XY Repeat .

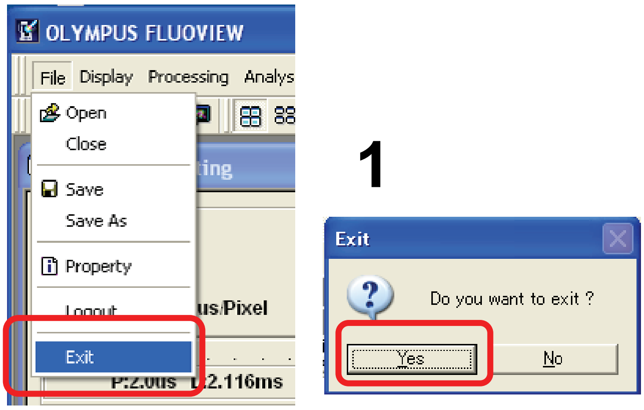
10. While observing the image, find the brightest fluorescent wavelength range by clicking the left slit.

11. Adjust the image.

12. To acquire the image, click the XYL button XY LZt .

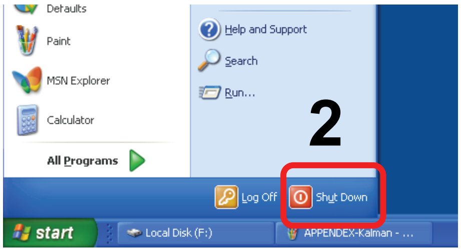
13. To save the λ image, click SeriesDone SeriesDone .

System Shutdown

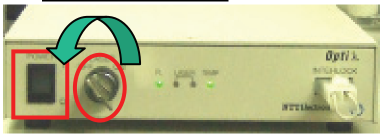

OLYMPUS FLUOVIEW
File Display Processing Analys
Open
Close
Save
Save As
Property
Logout
Exit
us/Pixel
P:2.0us L:2.116ms
1
Exit

Do you want to exit ?

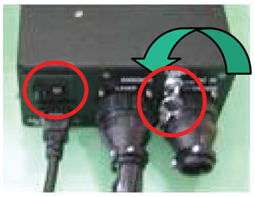
Yes
No


Defaults
Paint
MSN Explorer
Calculator
All Programs
Help and Support
Search
Run...
2
Log Off
Shut Down
start
Local Disk (F:)
APPENDEX-Kalman - ...

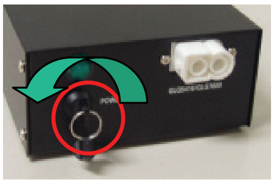
3 **B: LD559nm**

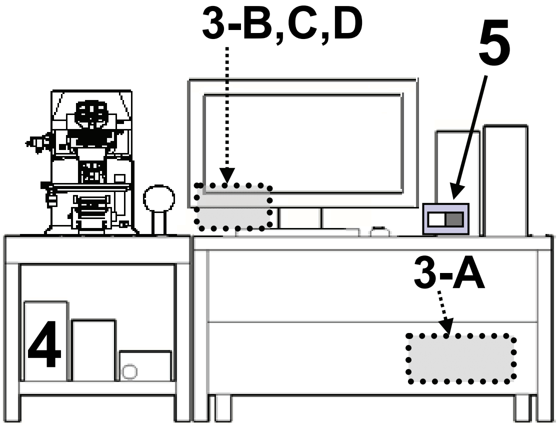


C: MAr laser



D: HeNe (G) laser





1. Exit the FV10-ASW software by selecting Exit from the File menu.

2. Shut down Windows.

3. Turn off the laser systems.

   A: MCPSU (405/440/473/635 nm lasers)
   Switch off the system.

   B: LD559nm
   Turn off the key switch to turn off the main power.

   C: Multi Ar laser
   Turn off the key switch to turn off the main power.

   D: HeNe (G) laser (543 nm)
   Turn off the key switch.

4. Switch off the power of the mercury lamp.

5. Switch off the central power.

Image Acquisition

— Applications —

XYT Image Acquisition

(Photobleaching with a single scanner)

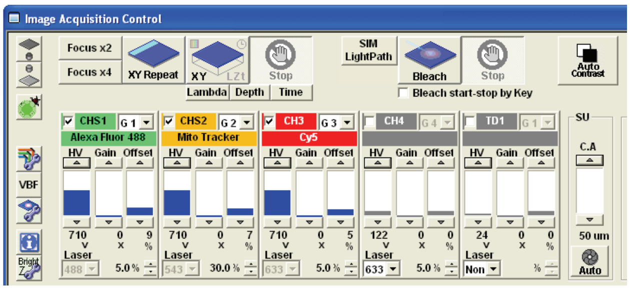
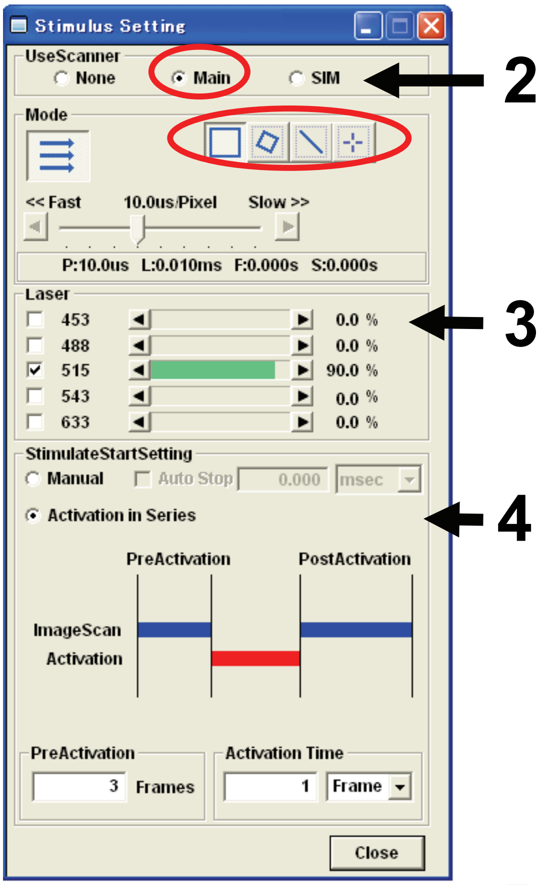


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
CHS1
Alexa Fluor 488
CHS2
Mito Tracker
CH3
Cy5
CH4
TD1
HV
Gain
Offset
Laser
VBF
Bright
SU
C.A
50 um
Auto

1. Make imaging adjustments.


Stimulus Setting
2
3
4
PreActivation
PostActivation
ImageScan
Activation
Close

UseScanner: ○ None ● Main ○ SIM

Mode

<< Fast 10.0us/Pixel Slow >>

P:10.0us L:0.010ms F:0.000s S:0.000s

Laser
- ☐ 453 0.0 %
- ☐ 488 0.0 %
- ☑ 515 90.0 %
- ☐ 543 0.0 %
- ☐ 633 0.0 %

StimulateStartSetting: ○ Manual ☐ Auto Stop 0.000 msec

● Activation in Series

PreActivation: 3 Frames

Activation Time: 1 Frame

2. Select Main in the Use Scanner window. Enclose the region to be photobleached using controls in the Mode section.

3. In the Laser section, select the photobleaching power.

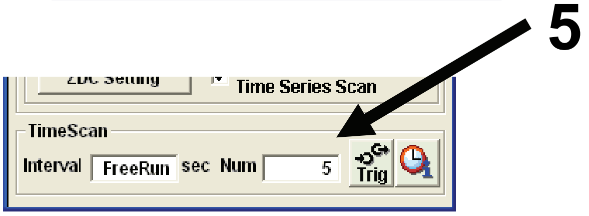
4. Set StimulateStartSetting.

When linking photobleaching with imaging

   4-1. Click the radio button for Activation in Series.

   4-2. Enter the PreActivation and Activation Time fields.

(For manual operation, refer to the following page.)


5
ZDC Setting
Time Series Scan
Trig

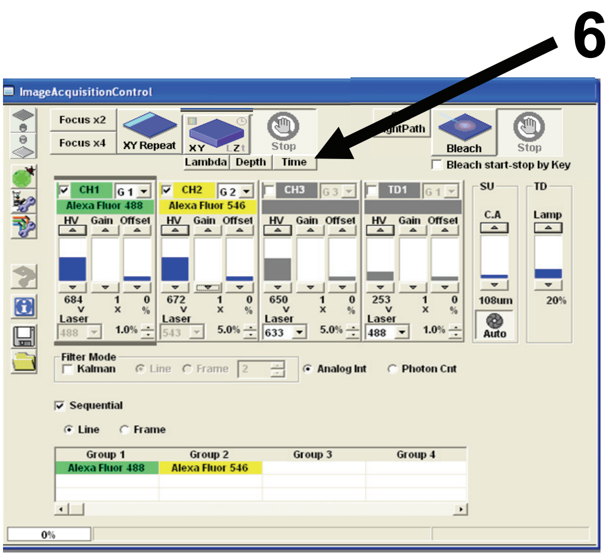
TimeScan

Interval FreeRun sec Num 5

5. Enter the time interval and number.

XYT Image Acquisition

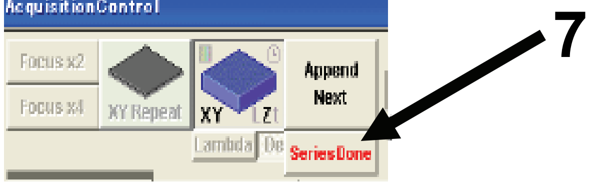
(Photobleaching with a single scanner)


6
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
Bleach
Stop
Bleach start-stop by Key
CH1
Alexa Fluor 488
CH2
Alexa Fluor 546
CH3
TD1
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Sequential
Line
Frame
Group 1
Group 2
Group 3
Group 4
0%

6. With Time selected, click the XYT button to execute image acquisition.

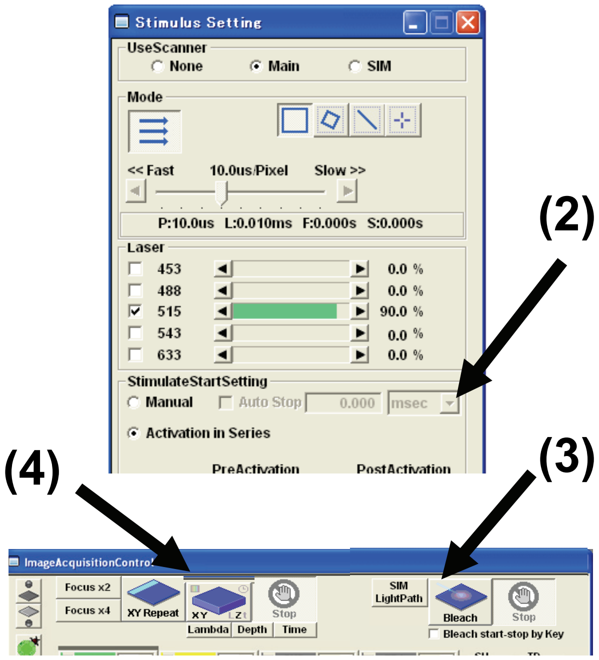
When linking photobleaching with imaging

The region is automatically photobleached at a set time.


7
AcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Append
Next
SeriesDone

7. Clicking SeriesDone causes the window bar of the acquired image to show “2DView-LiveImage(x).”

8. Save the image:
Save the image by selecting Save As from File on the menu bar.
(The Save As types “oib” and “oif” are the dedicated file formats for the FV10-ASW software.)


Stimulus Setting
UseScanner
None
Main
SIM
Mode
<< Fast
10.0us/Pixel
Slow >>
P:10.0us L:0.010ms F:0.000s S:0.000s
Laser
453
488
515
543
633
StimulateStartSetting
Manual
Auto Stop
msec
Activation in Series
PreActivation
PostActivation
(2)
(3)
(4)
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Stop
Bleach start-stop by Key

When photobleaching manually

(1) Perform operations 1 to 3 on the previous page.

(2) Click the radio button for Manual.
(To set the amount of time the Bleach button is enabled when it is clicked, check the checkbox for Auto Stop and enter the number of seconds.)

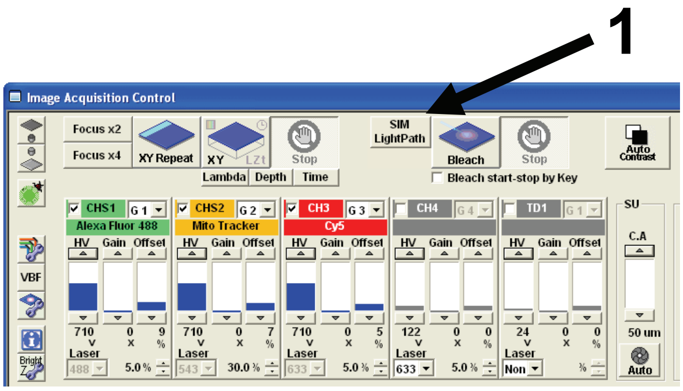
(3) Click the Bleach button.

(4) Observe the image in XY Repeat, XY, or XYT.

Only for SIM

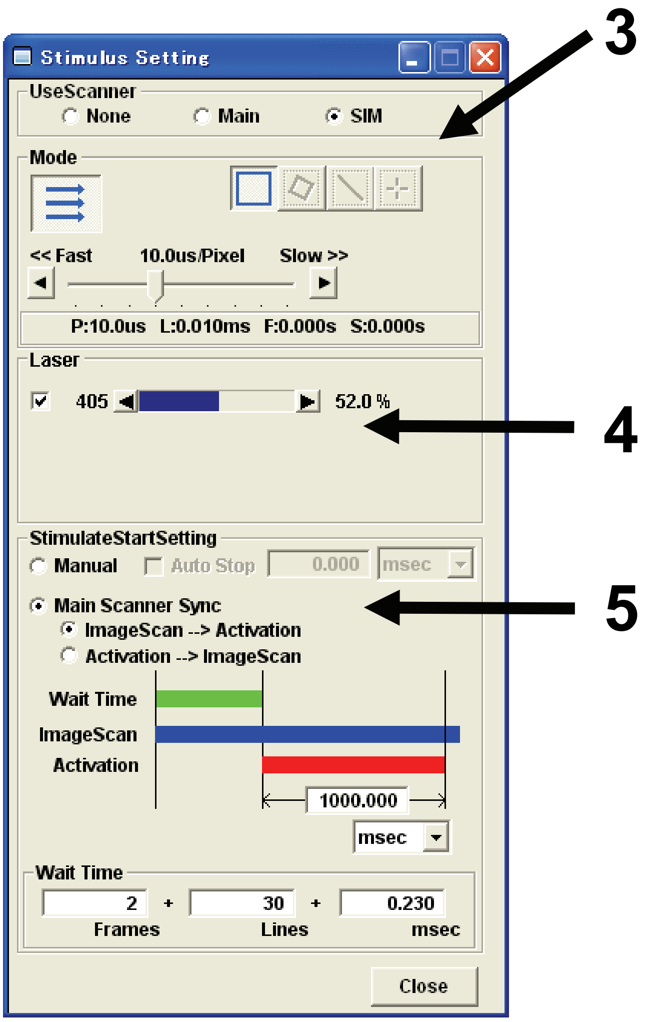
XYT Image Acquisition

(Photobleaching with a SIM scanner)


1
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
VBF
CH1
G1
Alexa Fluor 488
CH2
G2
Mito Tracker
CH3
G3
Cy5
CH4
G4
TD1
G1
HV
Gain
Offset
Laser
488
543
633
633
Non
5.0 %
30.0 %
5.0 %
5.0 %
SU
C.A
50 um
Auto

1. Click SIM LightPath.

2. Make imaging adjustments.


Stimulus Setting
3
4
5
Close

UseScanner: ○ None ○ Main ● SIM

Mode

<< Fast 10.0us/Pixel Slow >>

P:10.0us L:0.010ms F:0.000s S:0.000s

Laser: ☑ 405 52.0 %

StimulateStartSetting

○ Manual ☐ Auto Stop 0.000 msec

● Main Scanner Sync
  ● ImageScan --> Activation
  ○ Activation --> ImageScan

Wait Time
ImageScan
Activation
1000.000 msec

Wait Time
2 + 30 + 0.230
Frames Lines msec

3. Select SIM in the Use Scanner window. Enclose the region to be photobleached using controls in the Mode section.

4. In the Laser section, select the photobleaching power.

5. Set StimulateStartSetting.

When photobleaching manually

5-1. Click the radio button for Manual.

(To set the amount of time the Bleach button is enabled when it is clicked, check the checkbox for Auto Stop and enter the number of seconds.)

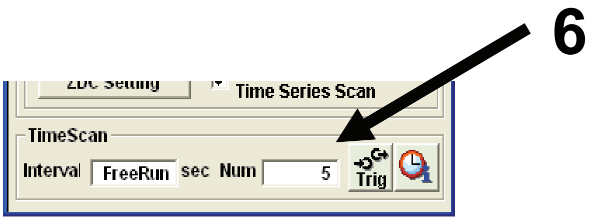
When linking photobleaching with imaging

5-1. Click the radio button for Main Scanner Sync and for ImageScan → Activation.

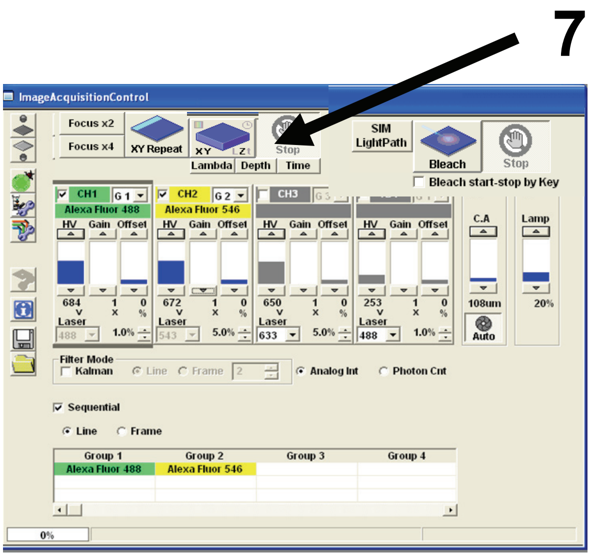
5-2. Enter the values for Activation and Wait Time.

XYT Image Acquisition

(Photobleaching with a SIM scanner)


6
Time Series Scan
TimeScan
Interval
FreeRun
sec
Num
5
Trig

6. Enter the time interval and number.


7
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
CH1
Alexa Fluor 488
CH2
Alexa Fluor 546
CH3
HV
Gain
Offset
Laser
C.A
Lamp
Auto
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Sequential
Line
Frame
Group 1
Group 2
Group 3
Group 4
Alexa Fluor 488
Alexa Fluor 546
0%

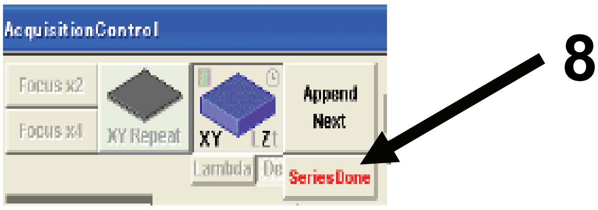
7. With Time selected, click the XYT button to execute image acquisition.

When photobleaching manually

Click the Bleach button when you want to start photobleaching.

When linking photobleaching with imaging

The region is automatically photobleached at the set time.


8
AcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Append
Next
SeriesDone

8. Clicking SeriesDone causes the window bar of the acquired image to show "2DView-LiveImage(x)."

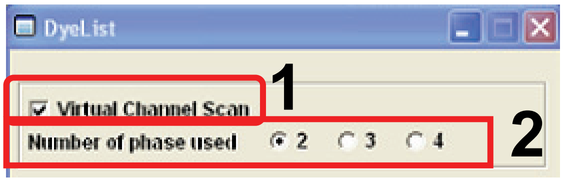
9. Save the image:
Save the image by selecting Save As from File on the menu bar.
(The Save As types "oib" and "oif" are the dedicated file formats for the FV10-ASW software.)

XY Image Acquisition

(Four-color staining with virtual channel)

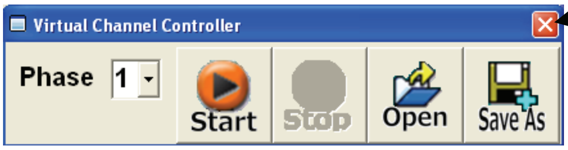
Acquiring a four-color staining image with virtual channel scan

Example: DAPI and Alexa488 for phase 1 / RodaminRed and Cy5 for phase 2

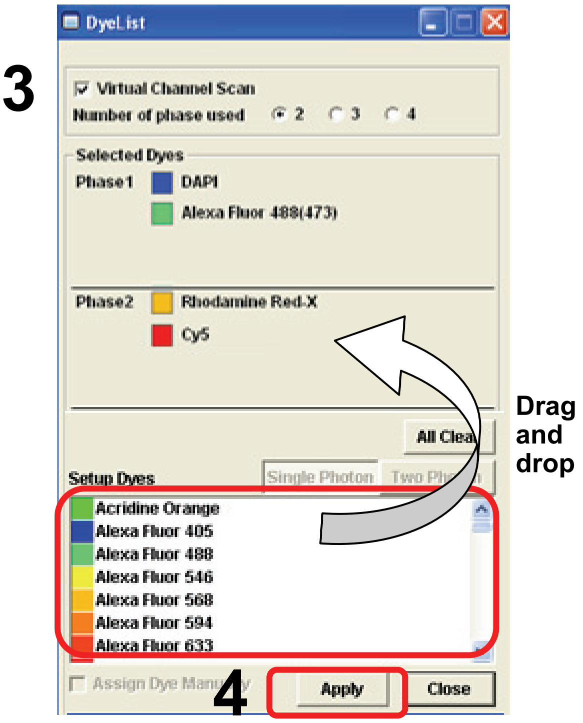


☑ Virtual Channel Scan
Number of phase used ● 2 ○ 3 ○ 4

1. Check the checkbox for ☑ Virtual Channel Scan in DyeList to automatically open the Virtual Channel Controller.


Virtual Channel Controller
Phase
1
Start
Stop
Open
Save As

2. In “Number of phase used,” select “2” as the phase count.


3
DyeList
Selected Dyes
All Clear
Setup Dyes
Single Photon
Two Photon
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488
Alexa Fluor 546
Alexa Fluor 568
Alexa Fluor 594
Alexa Fluor 633
Assign Dye Manually
4
Apply
Close
Drag and drop

☑ Virtual Channel Scan
Number of phase used ● 2 ○ 3 ○ 4

Phase1 🟦 DAPI
🟩 Alexa Fluor 488(473)

Phase2 🟧 Rhodamine Red-X
🟥 Cy5

3. Drag a dye from the dye list to each Phase.

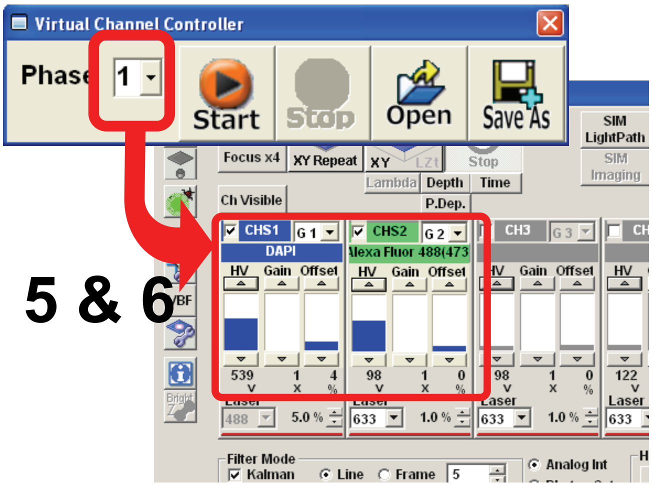
4. To set the fluorescent dyes and the phase count, click Apply.

5. Select Phase1 to display DAPI and Alexa488 in Image Acquisition Control.

   *** The slit width, filter, and DM settings are switched to DAPI and Alexa488.**

   Adjust the image.

6. Select Phase2 and adjust the image again.


5 & 6
Virtual Channel Controller
Phase
1
Start
Stop
Open
Save As
SIM LightPath
SIM Imaging
Focus x4
XY Repeat
XY
Lambda
Depth
Time
P.Dep.
Ch Visible
CH1 G1 DAPI
CH2 G2 Alexa Fluor 488(473)
CH3 G3
HV Gain Offset
539 1 4
98 1 0
122
Laser
488 5.0 %
633 1.0 %
Filter Mode
Kalman
Line
Frame
5
Analog Int

7. To acquire the image, click Virtual Channel Controller Start.

8. Save the image.

The use of this function does not reflect the LUT setting.

After image acquisition finishes, change the LUT setting by using LUT Setting .

HDRi Image Acquisition

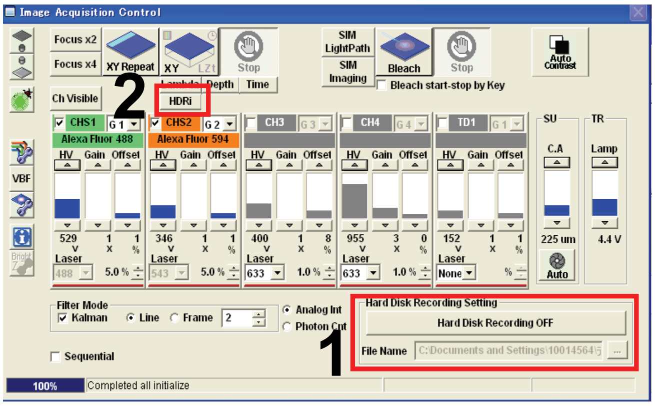


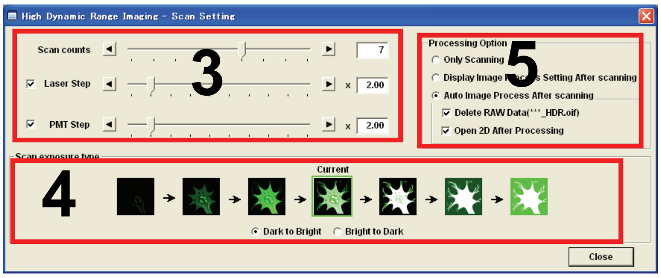
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
SIM LightPath
SIM Imaging
Bleach
Stop
Auto Contrast
☐ Bleach start-stop by Key
Ch Visible
HDRi
VBF
Bright Z
2
☑ CHS1 G 1
Alexa Fluor 488
☑ CHS2 G 2
Alexa Fluor 594
☐ CH3 G 3
☐ CH4 G 4
☐ TD1 G 1
HV Gain Offset
Laser
SU
C.A
TR
Lamp
Auto
1

CHS1: 529 V, 1 X, 1 %, Laser 488, 5.0 %
CHS2: 346 V, 1 X, 1 %, Laser 543, 5.0 %
CH3: 400 V, 1 X, 8 %, Laser 633, 1.0 %
CH4: 955 V, 3 X, 0 %, Laser 633, 1.0 %
TD1: 152 V, 1 X, 1 %, Laser None, %
C.A: 225 um
Lamp: 4.4 V

Filter Mode
☑ Kalman ● Line ○ Frame 2
● Analog Int
○ Photon Cnt
☐ Sequential

Hard Disk Recording Setting
Hard Disk Recording OFF
File Name C:\Documents and Settings\10014564\

100% Completed all initialize


High Dynamic Range Imaging - Scan Setting
3
5
Scan exposure type
4
Current
Close

Scan counts 7
☑ Laser Step x 2.00
☑ PMT Step x 2.00

Processing Option
○ Only Scanning
○ Display Image Process Setting After scanning
● Auto Image Process After scanning
☑ Delete RAW Data(***_HDR.oif)
☑ Open 2D After Processing

● Dark to Bright ○ Bright to Dark

HDRI Scan Setting window

1. After finishing image adjustment, set the save destination for hard disk recording.

2. Turn on HDRi in the Image Acquisition Control to open the HDRI Scan Setting window.

3. Set the condition for HDRi.
   - Scan count: number of scans (2 to 10)
   - Laser Step: increase/decrease rate of laser output
   - PMT Step: increase/decrease rate of HV

   *** The value of laser output in Acquisition Setting and the value of HV in Image Acquisition Control are applied to the basic values for HDRi.**

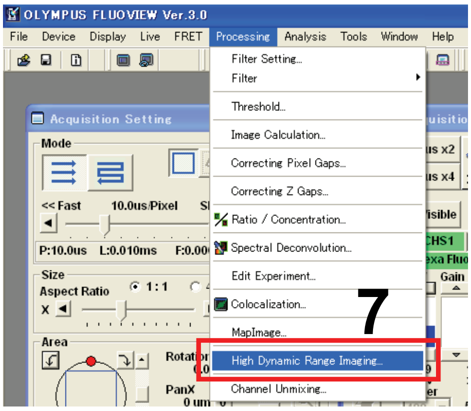
4. Select “Dark to Bright” or “Bright to Dark” and click the icon of acquisition sequence which is given the basic values for HDRi. “Current” is shown on the top of the selected icon.

Example of settings

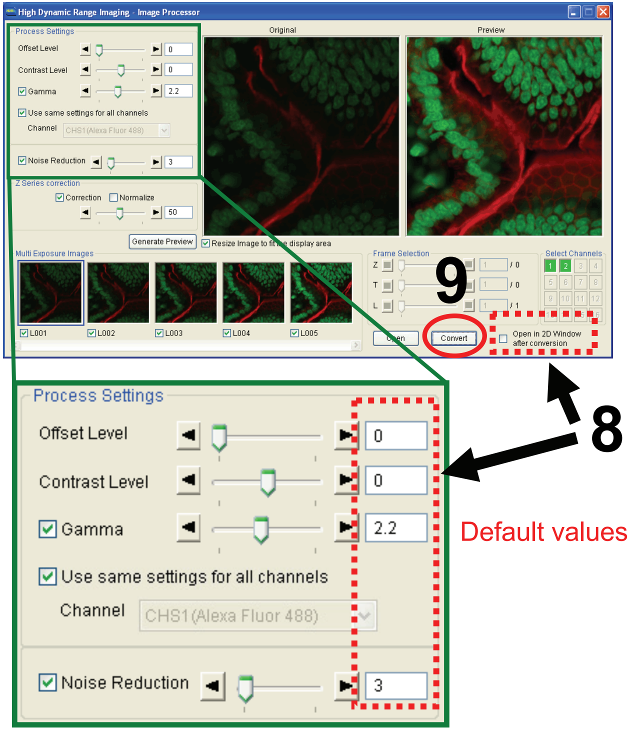
<Basic values>
Laser output in Acquisition Setting: 10.0 (%)
HV in Image Acquisition Control: 500 (V)
<Condition settings for HDRi >
Scan count = 5, Laser Step = x1.2, PMT Step = x1.2,
Dark to Bright, “Current” icon = 3rd from the left
<Setting results>
Laser output (%) : 6.9 -> 8.3 -> 10.0 -> 12.0 -> 14.4
HV (V) : 347 -> 417 -> 500 -> 600 -> 720

5. In the Processing Option section, set the process to be executed after image acquisition finishes.
   - **Only Scanning**: Only executes scanning.
   - **Display Image Process Setting After scanning**: Displays the HDRi Image Processor window after scanning finishes.
   - **Auto Image Process After scanning**: Executes HDRi image processing automatically after scanning finishes. (The parameters used when HDRi Image Processor was last used are applied.)

HDRi Image Acquisition


OLYMPUS FLUOVIEW Ver.3.0
File Device Display Live FRET Processing Analysis Tools Window Help
Acquisition Setting
Mode
Filter Setting...
Filter
Threshold...
Image Calculation...
Correcting Pixel Gaps...
Correcting Z Gaps...
Ratio / Concentration...
Spectral Deconvolution...
Edit Experiment...
Colocalization...
MapImage...
High Dynamic Range Imaging...
Channel Unmixing...
7

HDRi Image Processor window


High Dynamic Range Imaging - Image Processor
Original
Preview
Z Series correction
☑ Correction ☐ Normalize
Generate Preview
☑ Resize Image to fit the display area
Multi Exposure Images
☑ L001 ☑ L002 ☑ L003 ☑ L004 ☑ L005
Frame Selection
Select Channels
Open
Convert
☐ Open in 2D Window after conversion
9
8
Process Settings

Offset Level 0
Contrast Level 0
☑ Gamma 2.2
☑ Use same settings for all channels
Channel CHS1(Alexa Fluor 488)
☑ Noise Reduction 3

Default values

Offset Level: The intensity less then Offset value is regarded as 0. The low intensity noise on the image can be eliminated.
Contrast Level: The grayscale of entire images can be adjusted.
Gamma: Gamma correction.
Use same settings for all channels: If it is ticked, the same settings are performed in the image processing of all channels.
Noise Reduction: The noise can be reduced without losing the characteristics of the sample.

6. To start scanning images, click the XY button. Scanned images are saved in the set save destination for hard disk recording.
   (File names are assigned with the format ***_**HDR.oif**.)

When Auto Image Process After scanning is selected in step 6, images are saved after being automatically formatted in HDRI (Files named ***_.oif).

7. From the menu, select Processing and then High Dynamic Range Image to open the HDRi Image Processor window.

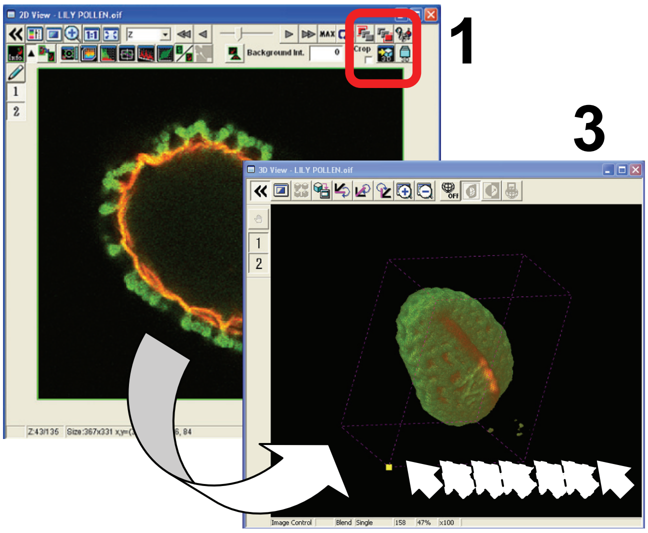
When Display Image Process Setting After scanning is selected in step 6, the window automatically opens.

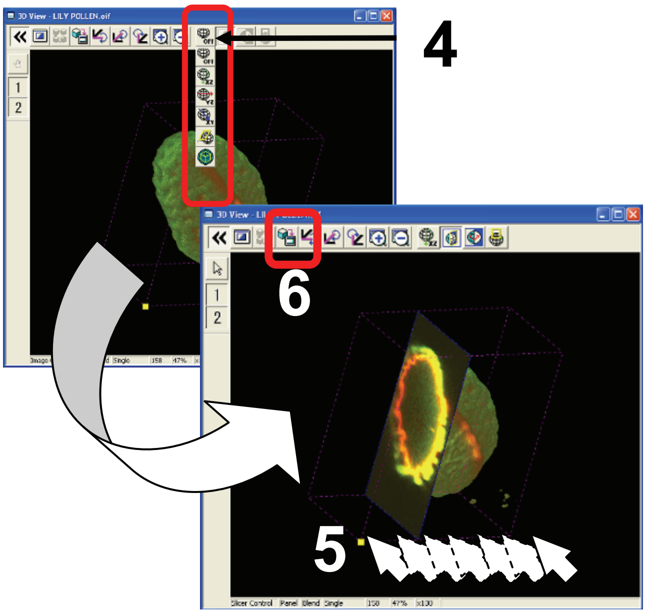
8. Set the Offset Level, Contrast Level, Gamma and Noise Reduction, and then check the checkbox of “Open in 2D Window after conversion”.

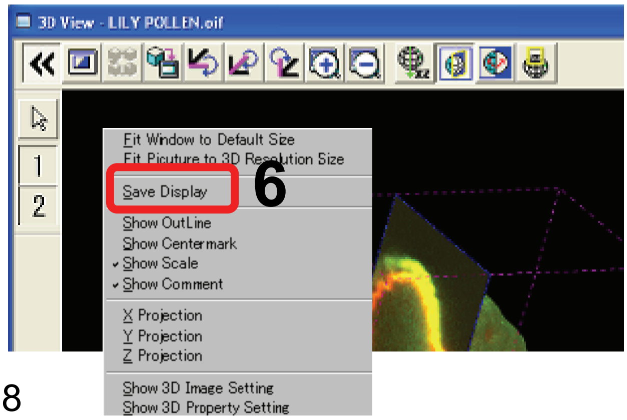
9. To create HDRi images, click the Convert button.

— Supplemental Operations —

3D Image Analysis 3D image creation/cross-sectional observation


2D View - LILY POLLEN.oif
1
3
3D View - LILY POLLEN.oif


3D View - LILY POLLEN.oif
4
6
5


3D View - LILY POLLEN.oif
Fit Window to Default Size
Fit Picuture to 3D Resolution Size
Save Display
6
Show OutLine
Show Centermark
Show Scale
Show Comment
X Projection
Y Projection
Z Projection
Show 3D Image Setting
Show 3D Property Setting

Observe an image from a desired angle.

1. Click 3D of the 2D View-(file name) image.

2. A 3D View is created.

3. To observe the object from a desired angle, move the 3D object by dragging the mouse on the image.

Observe the desired cross-section of an image.

4. Click OFF and then select XZ.

5. To observe the desired vertical cross-section, move the object right and left by dragging the mouse on the image.

Save the 3D image.

6. Click to create a 2D image. Save the image by right-clicking.

(Or, right click on the 3D image in order to select Save Display to save the view, including the ROI being displayed, in BMP format.)

3D Image Analysis Detailed settings for 3D image creation

Cut View button
Projects a cross-sectional view on a slice
when the slice graphics mode is set.

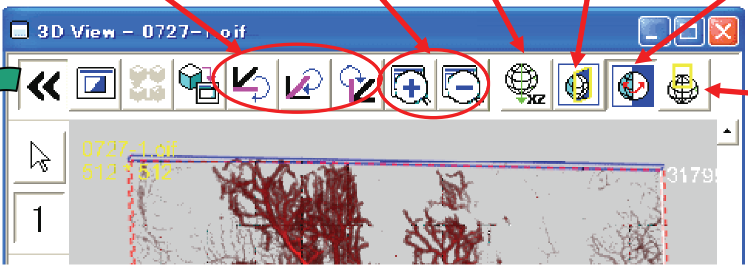
Image zoom in/out

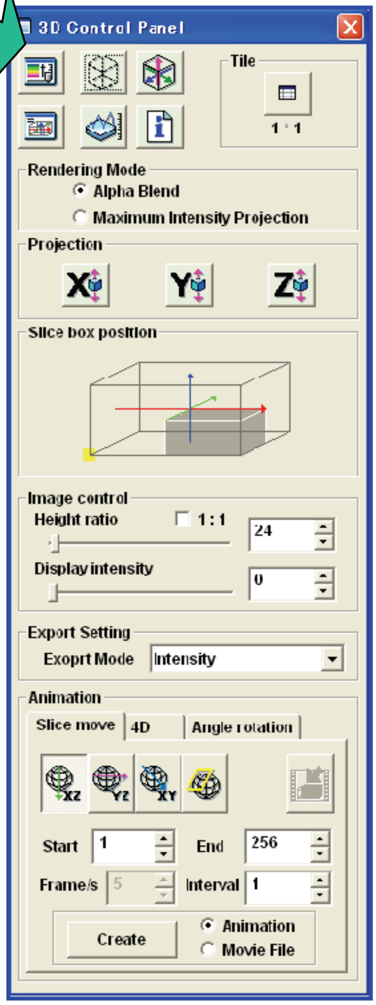
3D image rotation

Slice button

Change View Region button
Switches between the sliced 3D image
view region and the hidden region.

Orthogonal View button
Allows for observation with the view
point fixed at the front of a sliced
cross-section.


3D View – 0727-1.oif
0727-1.oif
512 * 512
1
3179


3D Control Panel
Tile
1 : 1
Projection
X
Y
Z
Slice box position
Animation
Slice move
4D
Angle rotation
XZ
YZ
XY
Create

Rendering Mode
● Alpha Blend
○ Maximum Intensity Projection

Image control
Height ratio ☐ 1 : 1 24
Display intensity 0

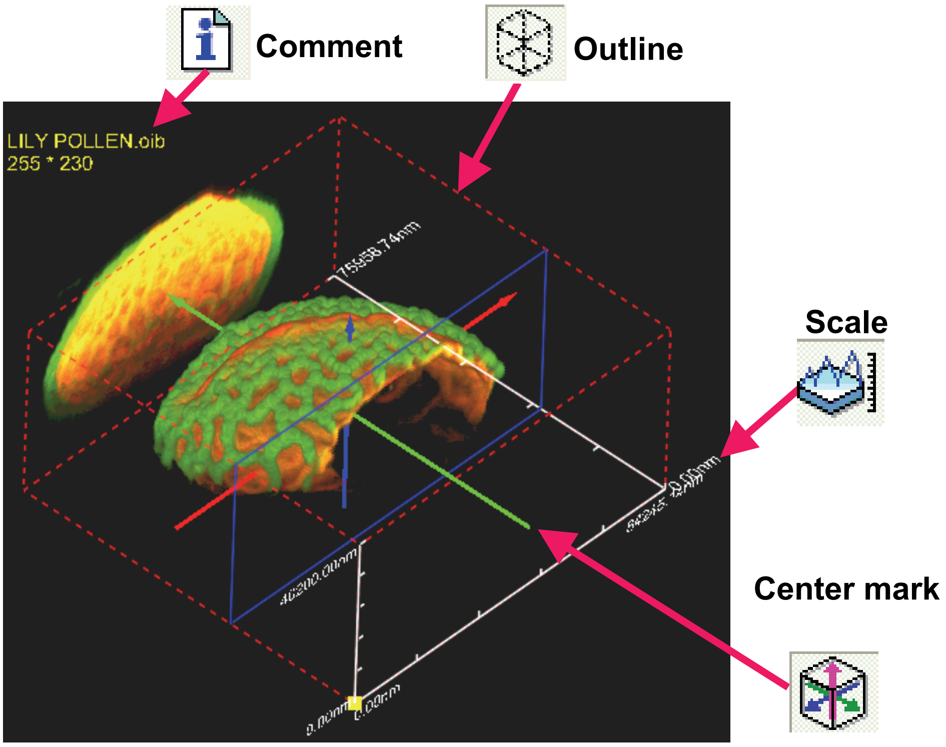
Export Setting
Exoprt Mode Intensity

Start 1 End 256
Frame/s 5 Interval 1
● Animation
○ Movie File

3D Image Settings (LUT adjustment, image adjustment, etc.)

3D Property Settings (image graphics setting such as scale bars and comments)

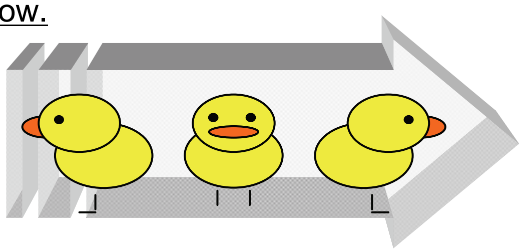
Graphics ON/OFF

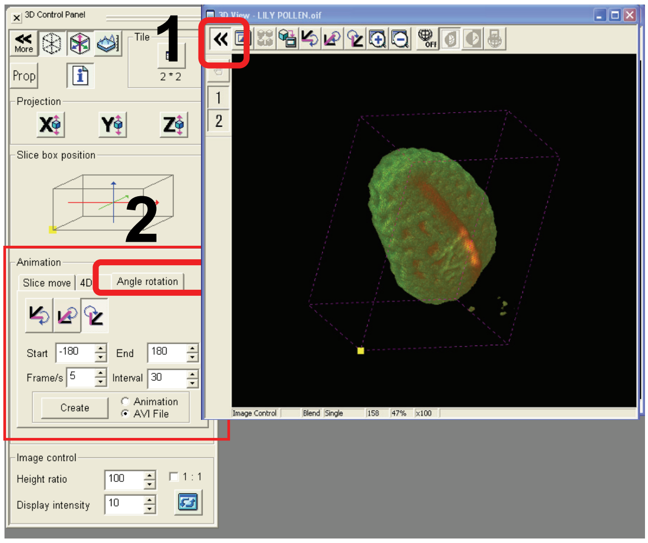

Comment
Outline
Scale
Center mark
LILY POLLEN.oib
255 * 230
75558.74nm
40200.00nm
54245.
0.00nm
0.00nm
0.00nm

3D Image Analysis How to create a file

To save a rotation file as a movie, render the image in 3D with the following procedure.

As an example, an image is rotated 180º below.

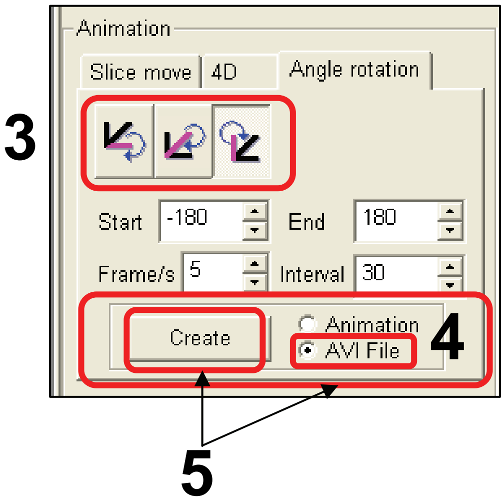




3D Control Panel
More
Tile
1
2 * 2
Prop
Projection
Slice box position
2
Animation
Slice move
4D
Angle rotation
Start -180
End 180
Frame/s 5
Interval 30
Create
Animation
AVI File
Image control
Height ratio 100
1 : 1
Display intensity 10
3D View - LILY POLLEN.oif

1. Click the « button.

2. Click the Angle rotation tab.



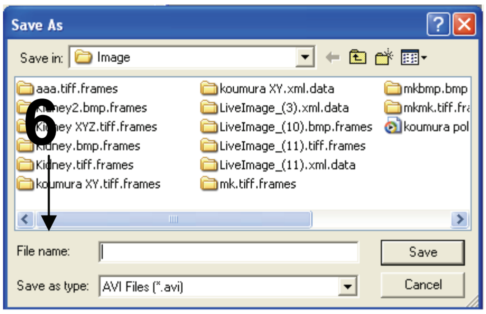
3
Animation
Slice move
4D
Angle rotation
Start -180
End 180
Frame/s 5
Interval 30
Create
○ Animation
● AVI File
4
5

3. Select the rotation axis.

4. Enter the rotation angle.

Start: starting angle / End: ending angle
Frame/s: rotation speed / Interval: angle interval

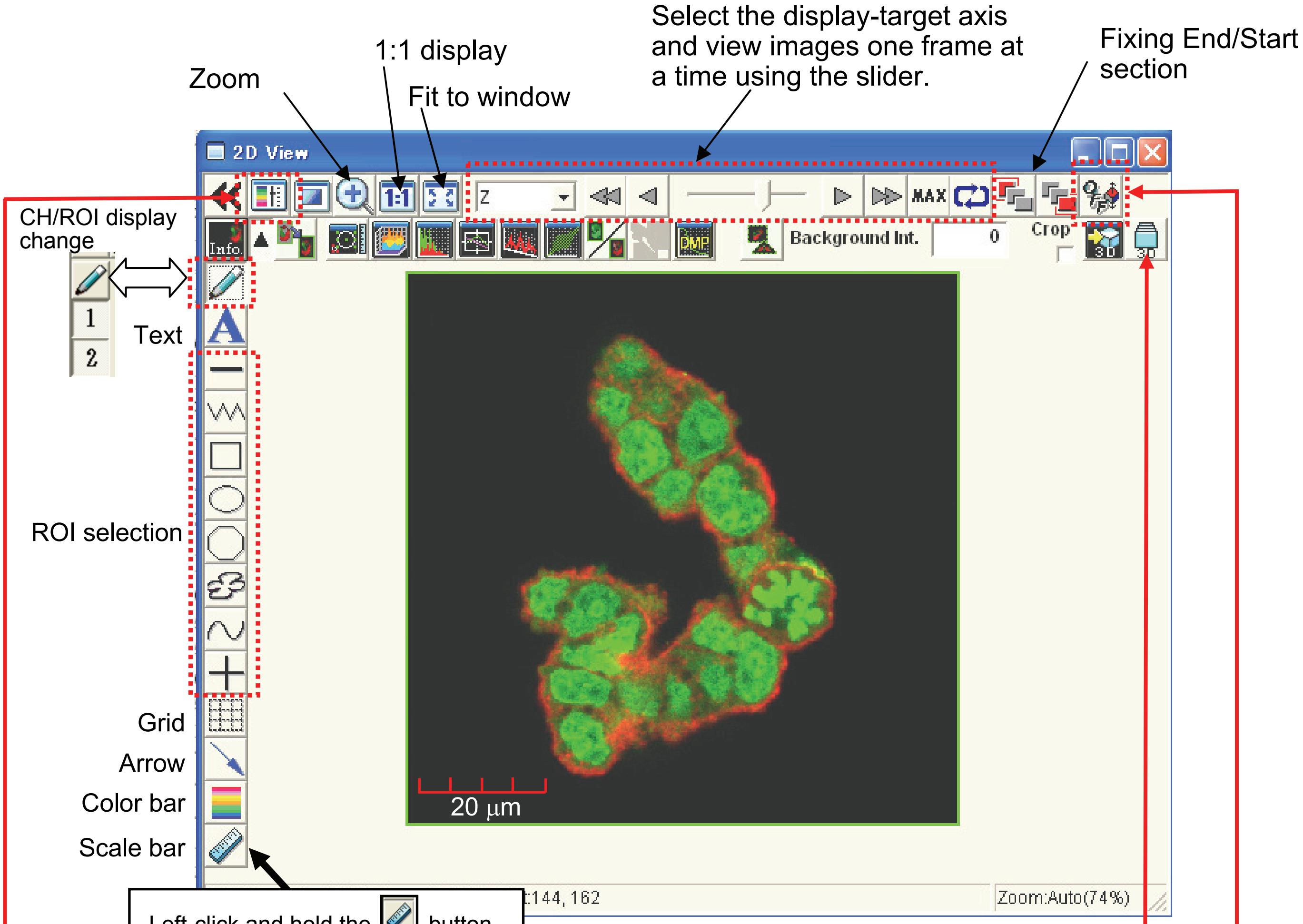
5. Select AVI File and then click Create.



Save As
Save in: Image
aaa.tiff.frames
koumura XY.xml.data
mkbmp.bmp
6
File name:
Save as type: AVI Files (*.avi)
Save
Cancel

6. Enter a file name and then click Save.

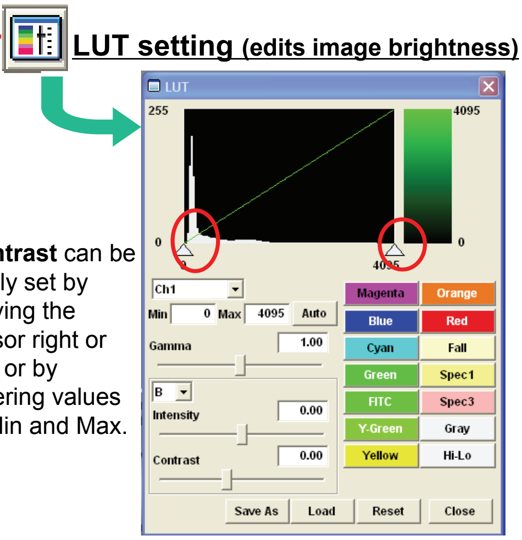
2D View Window


Zoom
1:1 display
Fit to window
Fixing End/Start section
CH/ROI display change
1
2
Text
ROI selection
Grid
Arrow
Color bar
Scale bar
2D View
Z
MAX
Info.
Background Int.
0
Crop
3D
20 µm
:144, 162
Zoom:Auto(74%)

Select the display-target axis and view images one frame at a time using the slider.

Left-click and hold the button to drag it to a place on the image and drop it there.
(A straight line can be drawn by pressing Shift when left-clicking.)

LUT setting (edits image brightness)


LUT
255
4095
0
0
4095
Auto
Magenta
Orange
Blue
Red
Cyan
Fall
Green
Spec1
FITC
Spec3
Y-Green
Gray
Yellow
Hi-Lo
Save As
Load
Reset
Close

Ch1

Min 0 Max 4095

Gamma 1.00

B

Intensity 0.00

Contrast 0.00

Contrast can be freely set by moving the cursor right or left, or by entering values in Min and Max.

Creation of rotating 3D animation

Creates a rotating 3D animation based on the X axis (standard animation).

Creates a rotating 3D animation based on the Y axis (standard animation)

Displays the 3D Animation window that can be used to customize the settings of the rotating 3D animation creation process.
Enter settings and click New Image to create a 3D animation.

Projection mode change

Projection Off button: No projection displayed.

Z Intensity Projection button: Displays Z Intensity Projection images.

T Intensity Projection button: Displays T Intensity Projection images.

λ Intensity Projection button: Displays λ Intensity Projection images.

TOPO Z Topographic Projection button: Displays Z Topographic Projection images.

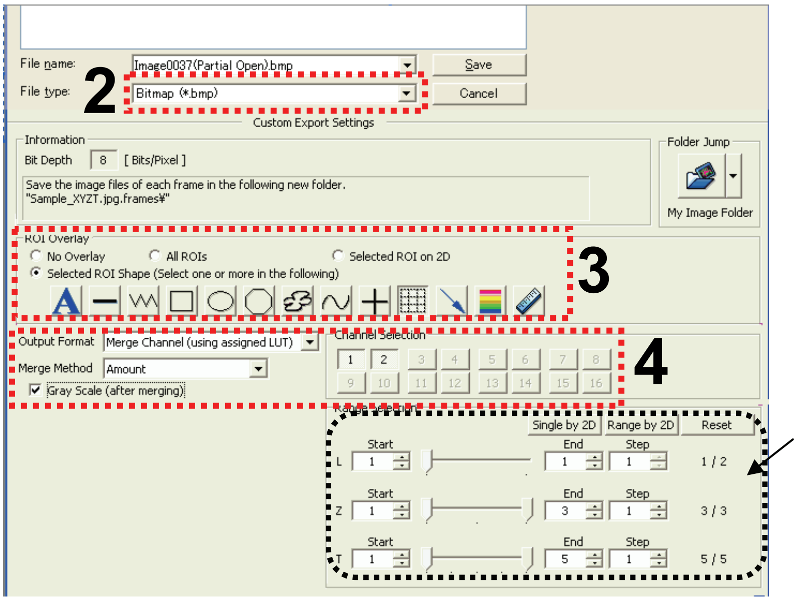
L TOPO λ Topographic Projection button: Displays λ Topographic Projection images.

T Series Average button: Displays T series average images.

Acquired Image Export

Saving images as Bitmap (*.bmp), JPEG (*.jpg, *.jpeg), TIFF (*.tif, *.tiff), or PNG (*.png)

1. Open the Live View window or 2D View window of the image to be saved. Right-click on the image and then select Export.
2. Select a file format to use for the export.
3. When saving a ROI in an image, such as a scale bar, select All ROIs or Selected ROI Shape.
4. Select the output mode of the image. In Channel Selection, select an output channel.
    - Merging images
    - LUT images for each CH
    - Raw data (black and white) images
5. To save the file, click Save.


File name: Image0037(Partial Open).bmp

2 File type: Bitmap (*.bmp)

Custom Export Settings

Information

Bit Depth 8 [Bits/Pixel]

Save the image files of each frame in the following new folder.
"Sample_XYZT.jpg.frames¥"

3 ROI Overlay: ○ No Overlay ○ All ROIs ○ Selected ROI on 2D ● Selected ROI Shape (Select one or more in the following)

4 Output Format: Merge Channel (using assigned LUT)

Merge Method: Amount

☑ Gray Scale (after merging)

Channel Selection: 1 2

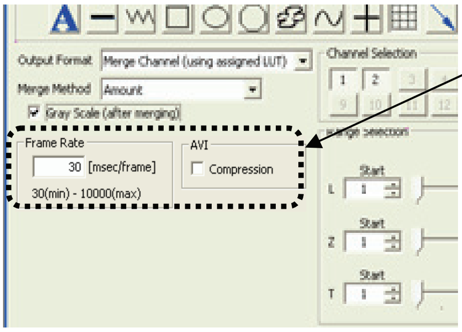
Range selection

	Start	End	Step	
L	1	1	1	1 / 2
Z	1	3	1	3 / 3
T	1	5	1	5 / 5

Range selection
Specify the Start and End values of axes to be read (L/Z/T).

Saving images in movie format (AVI or MOV) (The AVI or MOV format is available for XYZ, XYT, XYλ, XZT, XλT, and XλZ images.)

1. Open the Live View window or 2D View window of the image to be saved. Right-click on the image and then select Export.
2. Select a movie format and enter the frame rate in the Frame Rate field.
3. To save the file, click Save.


Output Format: Merge Channel (using assigned LUT)

Merge Method: Amount

☑ Gray Scale (after merging)

Frame Rate: 30 [msec/frame] 30(min) - 10000(max)

AVI: ☐ Compression

Frame Rate
The frame rate of the movie and whether to set compression or not can be set.
(AVI: Sets whether to compress the AVI file.)

Only for SPD

Adjustment of Acquisition Wavelength Range (VBF)

The wavelength range of waves acquired in Ch1 and Ch2 can be adjusted by using the **Variable Barrier Filter**.

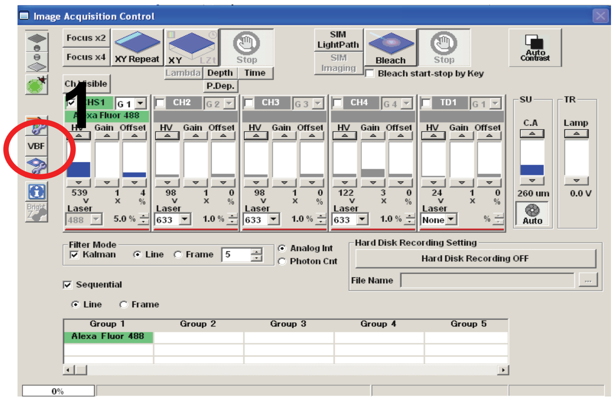
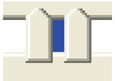
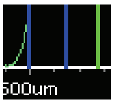
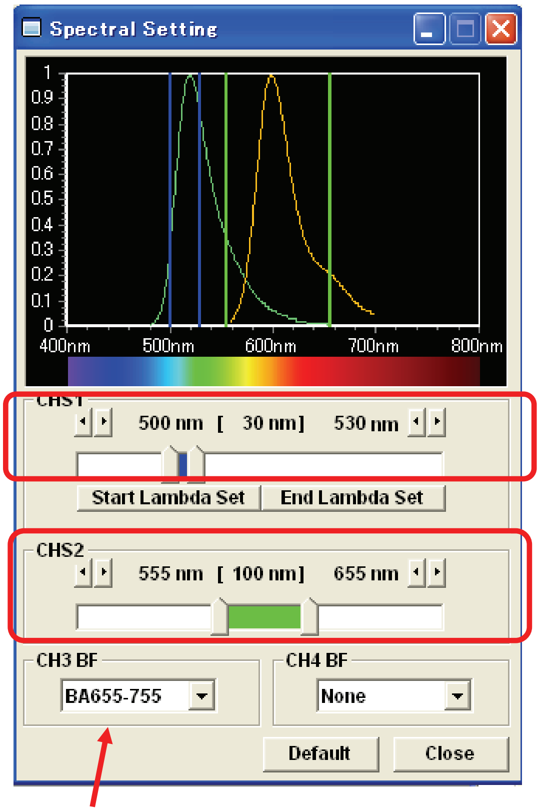


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
P.Dep.
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
VBF
1
CHS1
CH2
CH3
CH4
TD1
SU
TR
Alexa Fluor 488
HV
Gain
Offset
C.A
Lamp
Auto
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Hard Disk Recording Setting
Hard Disk Recording OFF
File Name
Sequential
Line
Frame
Group 1
Group 2
Group 3
Group 4
Group 5
Alexa Fluor 488

1. Click the **VBF** button to open the Spectral Setting window.

2. Using  or , move the position to adjust the wavelength of the wave to be acquired.

Spectral Setting window


Spectral Setting
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400nm
500nm
600nm
700nm
800nm
Start Lambda Set
End Lambda Set
Default
Close

CHS1
500 nm [30 nm] 530 nm

CHS2
555 nm [100 nm] 655 nm

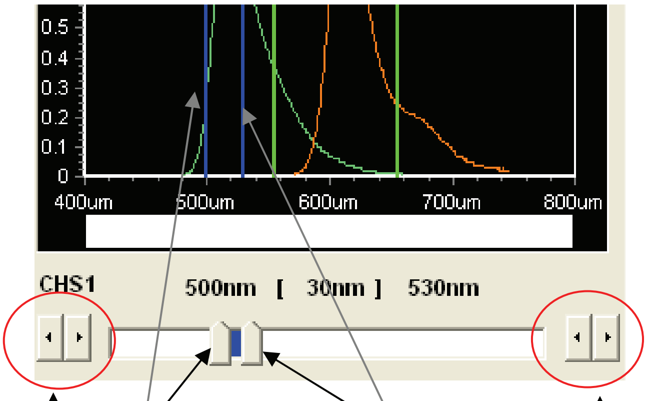
CH3 BF
BA655-755

CH4 BF
None

Slit adjustment for Ch1

Slit adjustment for Ch2

Fluorescent filter selection for Ch3


0.5
0.4
0.3
0.2
0.1
0
400um
500um
600um
700um
800um

CHS1
500nm [30nm] 530nm

Changes the **slit position**.

Changes the **slit width**.

Changes the **slit position** in 1 nm increments.

Changes the **slit width** in 1 nm increments.

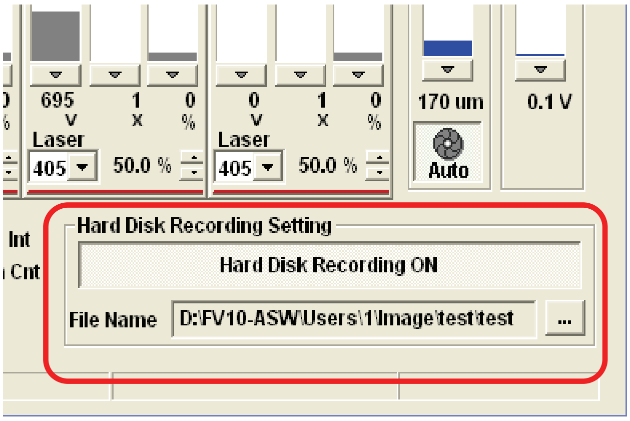
Hard Disk Recording

- This function automatically saves acquired images in a preset save destination.
- When series imaging is executed, you can check images up to the immediately preceding one through the Viewer window in the middle of the image acquisition process.

[Hard disk recording conditions]

Maximum number of image acquisition	32,766 times (number of images in series)
Save destination	Only local drives are guaranteed (D drive)
Other conditions	XYT images below 128×128 in size. Intervals of 1 second or more are required. Disabled for the reciprocating scan mode. **The review function is only available for interval operation.**

How to acquire images



File Name D:\FV10-ASW\Users\1\Image\test\test

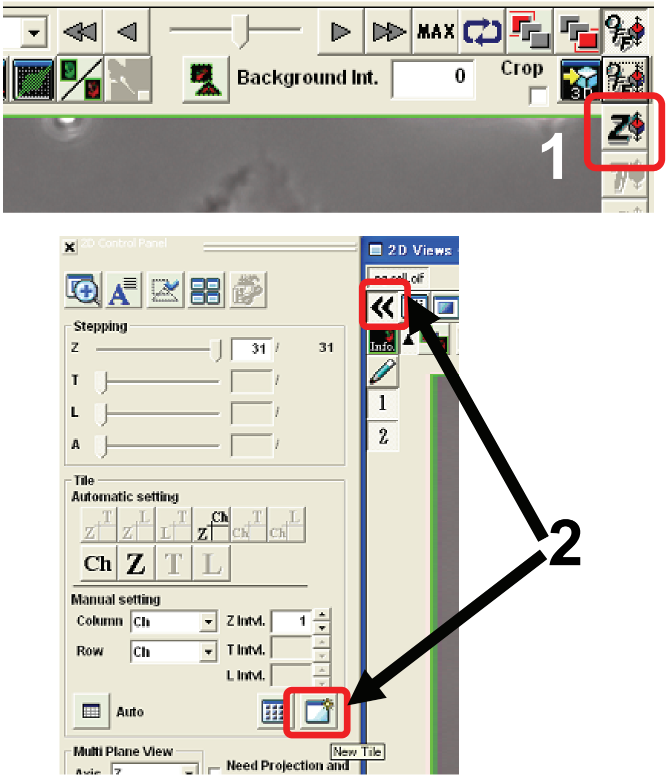
1. Set the image adjustment and condition settings.
2. Click the Hard Disk Recording OFF button. The button appears as Hard Disk Recording On and the Viewer window opens.
3. Click the ... button next to the File Name textbox. Set the save destination and file name and then click Open.
4. To acquire images, click the XY LZt button.

Viewing images in the middle of the image acquisition process

1. After image acquisition starts, bring the Viewer window to the front.
2. Double-click an image file during the acquisition process with the view set to Thumbnail in the Explorer window.

(When image files are not displayed in the Thumbnail view during the acquisition process, right-click and select Refresh.)

A. Creation of 2D Z Projection Images
B. Reloading Set Conditions

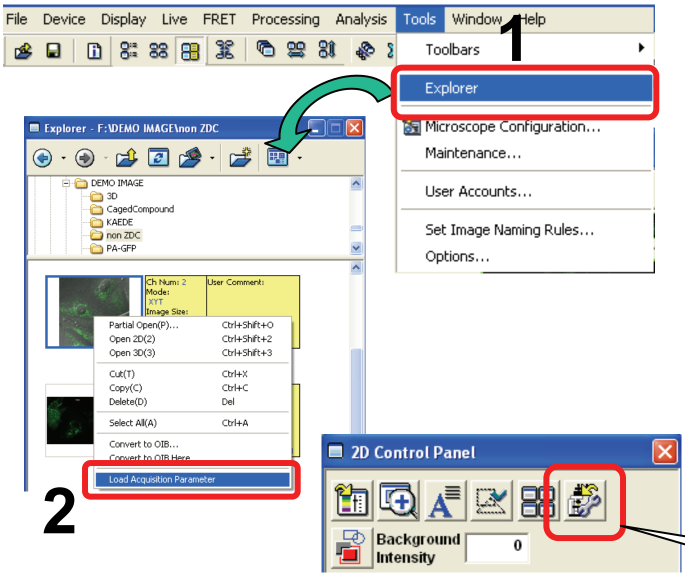

MAX
Background Int.
0
Crop
1
2D Control Panel
2D Views
Stepping
Z
31
31
T
L
A
Tile
Automatic setting
Ch
Z
T
L
Manual setting
Column
Ch
Z Intvl.
1
Row
Ch
T Intvl.
L Intvl.
Auto
New Tile
Multi Plane View
Need Projection and
Info.
1
2
2

A. Creation of 2D Z projection images

1. Open an original image and then select Z from the Projection button in the 2D View to open the Z projection image.

2. Click the New Tile button in the 2D Control Panel (opened by clicking «) to create a 2D image of the Z projection image.

3. Save the image.


File
Device
Display
Live
FRET
Processing
Analysis
Tools
Window
Help
Toolbars
1
Explorer
Microscope Configuration…
Maintenance…
User Accounts…
Set Image Naming Rules…
Options…
Explorer - F:\DEMO IMAGE\non ZDC
DEMO IMAGE
3D
CagedCompound
KAEDE
non ZDC
PA-GFP
Ch Num: 2
Mode:
XYT
Image Size:
User Comment:
Partial Open(P)…
Ctrl+Shift+O
Open 2D(2)
Ctrl+Shift+2
Open 3D(3)
Ctrl+Shift+3
Cut(T)
Ctrl+X
Copy(C)
Ctrl+C
Delete(D)
Del
Select All(A)
Ctrl+A
Convert to OIB…
Convert to OIB Here
Load Acquisition Parameter
2
2D Control Panel
Background Intensity
0

B. Reloading set conditions

Loads image acquisition conditions from past image data in order to duplicate them.

1. Select Explorer from Tools.

2. Right-click on a thumbnail image from which to reload set conditions, and then select Load Acquisition Parameter.

3. The conditions are reloaded.

Conditions can be reloaded by clicking the Load Scan Data button while the image from which to reload conditions is open.

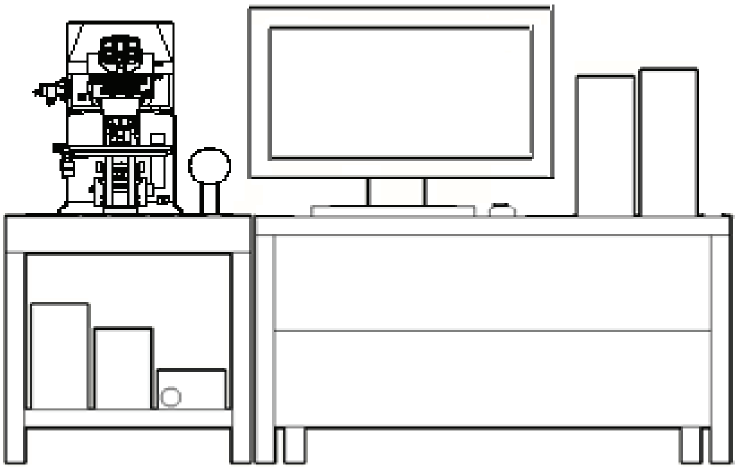
OLYMPUS®

Laser Scanning Biological Microscope

FV1200

BX61WI

Quick Start



FV1200 BX61WI
Quick Start
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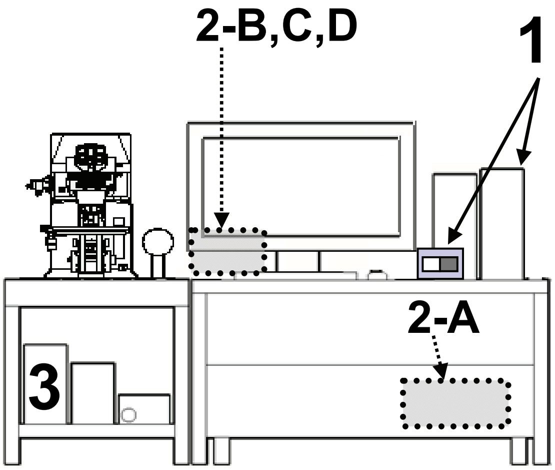
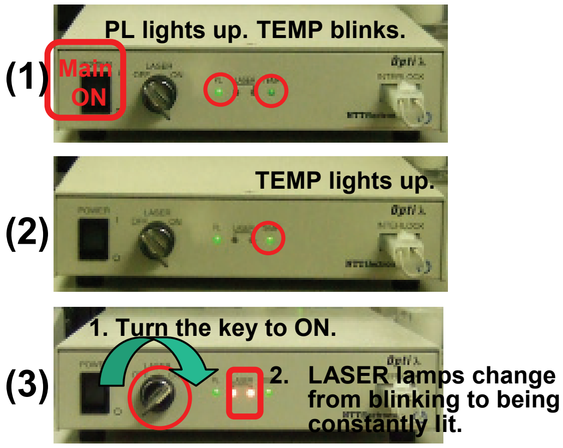
Image Acquisition

— Fundamentals —

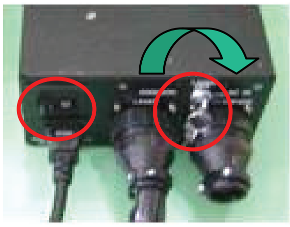
System Startup



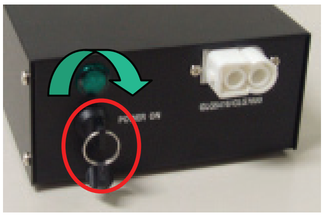
B: LD559 laser


(1)
PL lights up. TEMP blinks.
Main ON
(2)
TEMP lights up.
(3)
1. Turn the key to ON.
2. LASER lamps change from blinking to being constantly lit.

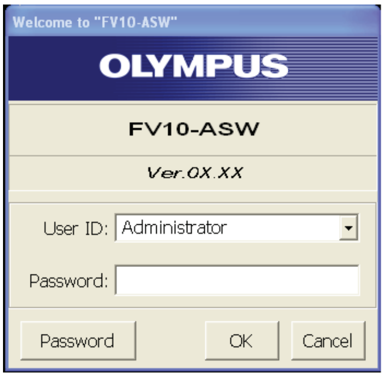
C: MAr laser



D: HeNe (G) laser



5


Welcome to "FV10-ASW"
OLYMPUS
FV10-ASW
Ver.0X.XX
Password
OK
Cancel

User ID: Administrator

Password:

1. Switch on the central power and then start up the Controller.

2. Start up the laser systems.

A: MCPSU (405/440/473/559/635 nm)

Switch on the system.

B: LD559

(1) Switch on the main power of the laser system. The PL lamp lights up in **green** and the TEMP lamp blinks in **green**.

(2) Confirm that the TEMP lamp has changed from blinking to being constantly lit.

(3) Turn on the key switch. The LASER lamps **blink in red**. It takes a while for the lasers to stabilize (The lamps change from **blinking in red** to being constantly **lit in red**). After the LASER lamps are **lit in red**, the LD559 laser system is ready for use.

C: MultiAr laser

Switch on the main power and then turn the key switch clockwise.

D: HeNe (G) laser (543 nm)

Turn the key switch clockwise.

3. Switch on the power of the mercury lamp unit. *** Used for visual observation**

4. Enter the user name and password to log on to Windows.

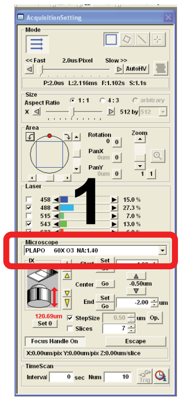
OS	User name	Passwprd
Windows7 (64bit)	olympus	olympus
Others	fluoview	fluoview

5. Double click FV to start up the FV10-ASW software.

User name: Administrator
Password: Administrator

Visual Observation via Microscope

■ ■ Fluorescent image observation ■ ■


AcquisitionSetting
Mode
Size
Area
Laser
Microscope
PLAPO 60X O3 NA:1.40
TimeScan
1

■ Memo ■

Fluorescent mirror unit

WBV: blue violet excitation (ECFP, etc.)

NIBA: blue excitation/green fluorescence (Example: FITC, EGFP, etc.)

WIG: green excitation/red fluorescence (Example: rhodamine, DsRed, etc.)

WU: UV excitation

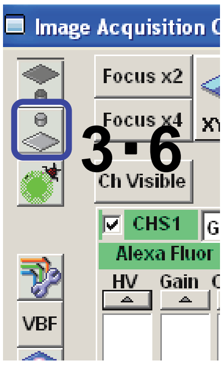
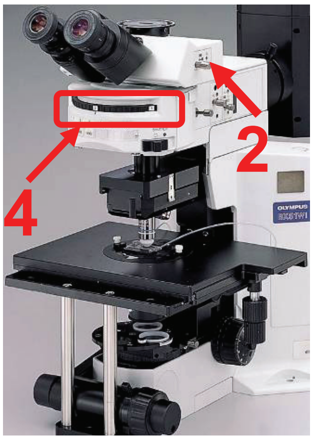
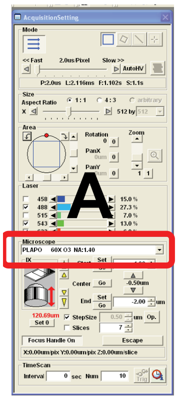


Image Acquisition C
Focus x2
Focus x4
XY
Ch Visible
CHS1
Alexa Fluor
HV
Gain
VBF
3･6


2
4
OLYMPUS
BX51WI

1. Set objective lenses on the microscope and select an objective lens in the FV10-ASW software.
2. Set the light path selector knob of the trinocular tube at the position of [◁ (eye)].
3. Click in the FV10-ASW software.
4. Select a fluorescent mirror unit. (Refer to the Memo.)
5. Adjust the focus.
6. After observation finishes, click to close the shutter of the fluorescent lamp.

■ ■ DIC image observation ■ ■


AcquisitionSetting
Mode
Size
Area
Laser
Microscope
PLAPO 60X O3 NA:1.40
TimeScan
A

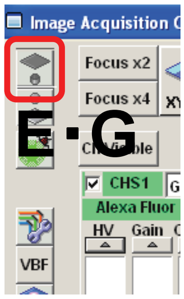
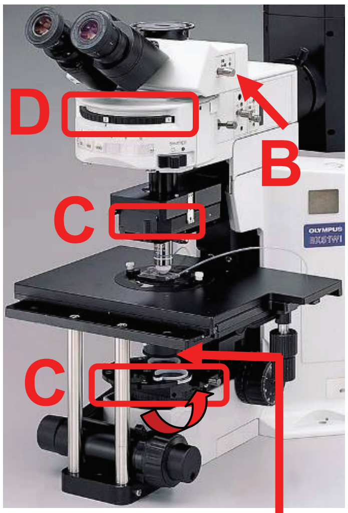


Image Acquisition C
Focus x2
Focus x4
XY
Ch Visible
CHS1
Alexa Fluor
HV
Gain
VBF
E･G


D
B
C
C
OLYMPUS
BX51WI

This model is the BX51WI

A. Set objective lenses on the microscope and select an objective lens in the FV10-ASW software.

B. Set the light path selector knob of the trinocular tube at the position of [◁ (eye)].

C. Insert the polarizer in the optical axis and select the DIC elements on both sides of the objective lens and condenser.

D. Select the [DICT] analyzer in the fluorescent mirror turret.

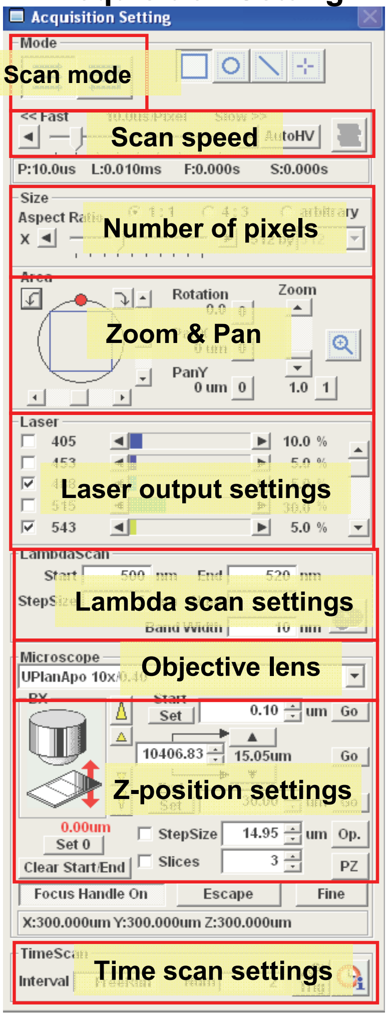
E. Click in the FV10-ASW software.

F. Adjust the focus.

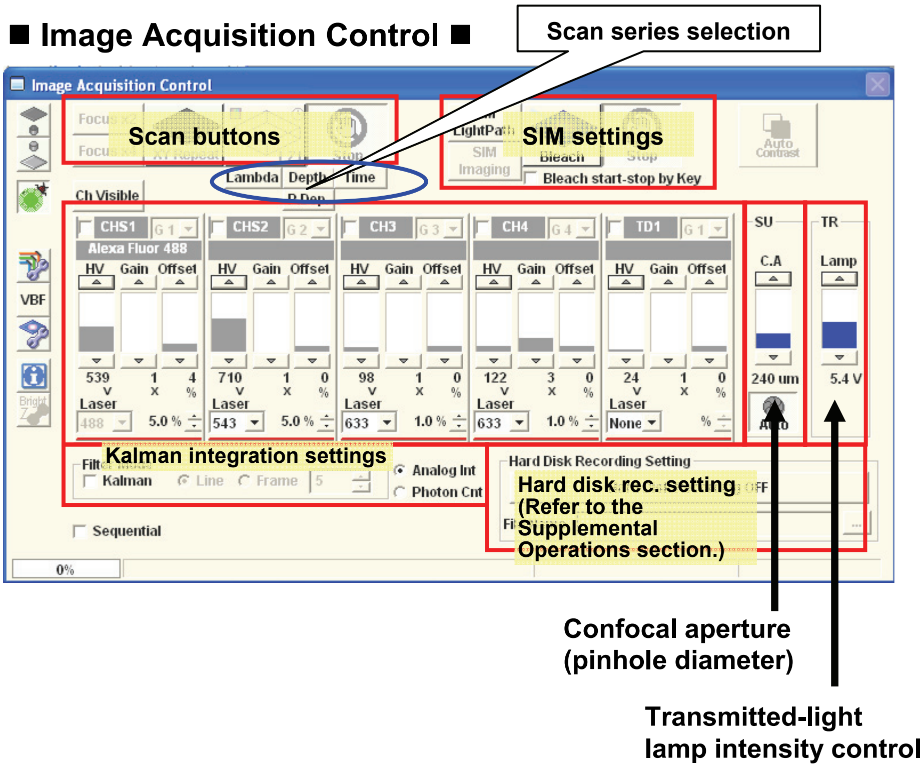
G. After observation finishes, click to close the shutter of the halogen lamp.

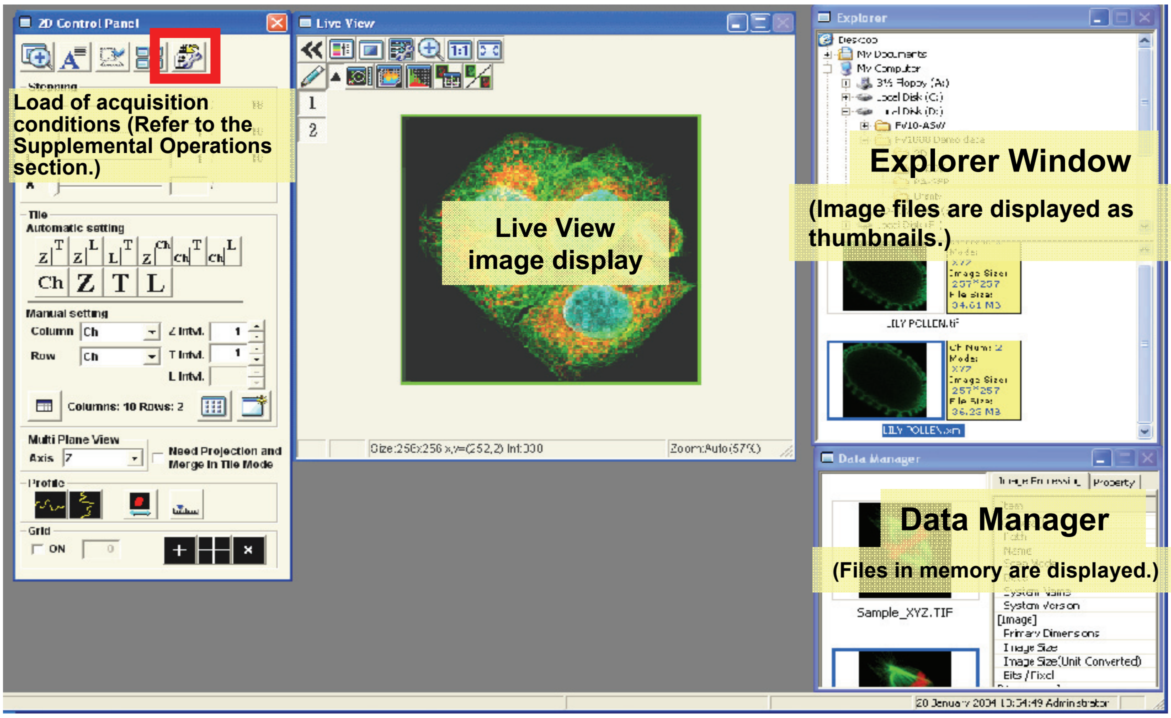
Overview of Image Acquisition Panel

■ Acquisition Setting ■


Scan mode
Scan speed
Number of pixels
Zoom & Pan
Laser output settings
Lambda scan settings
Objective lens
Z-position settings
Time scan settings

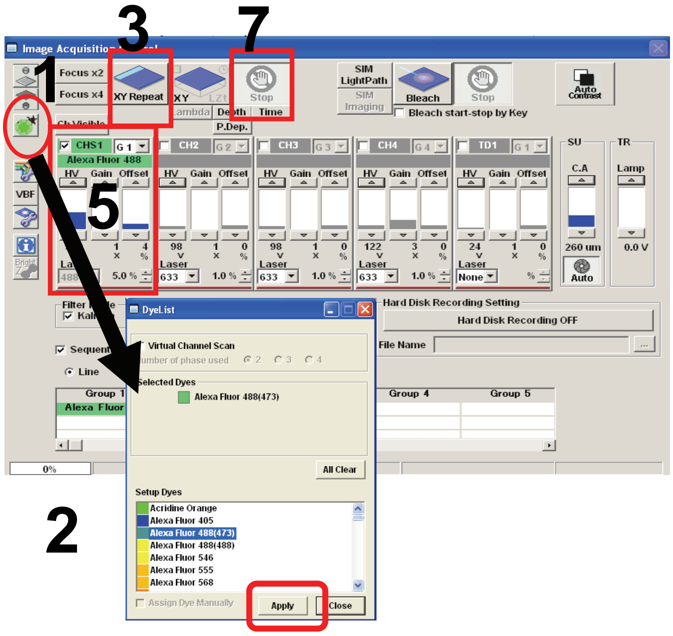
■ Image Acquisition Control ■


Scan series selection
Scan buttons
SIM settings
Kalman integration settings
Hard disk rec. setting
(Refer to the
Supplemental
Operations section.)
Confocal aperture
(pinhole diameter)
Transmitted-light
lamp intensity control

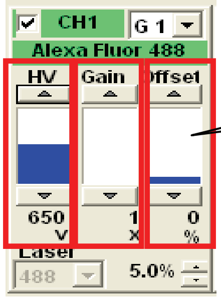

Load of acquisition
conditions (Refer to the
Supplemental Operations
section.)
Live View
image display
Explorer Window
(Image files are displayed as
thumbnails.)
Data Manager
(Files in memory are displayed.)

XY Image Acquisition (Single-color staining)

Example: single-color staining with green fluorescence (Alexa488)


1
2
3
5
7
Image Acquisition
Focus x2
Focus x4
XY Repeat
XY
Stop
Depth
Time
P.Dep.
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
CHS1
Alexa Fluor 488
CH2
CH3
CH4
TD1
HV
Gain
Offset
Laser
SU
TR
C.A
Lamp
260 um
0.0 V
Auto
Hard Disk Recording Setting
Hard Disk Recording OFF
File Name
DyeList
Virtual Channel Scan
Selected Dyes
Alexa Fluor 488(473)
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close
Sequential
Line
Group 1
Group 4
Group 5

5


CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650
V
1
X
0
%
Laser
488
5.0%

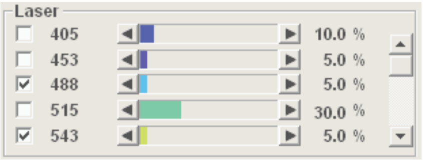
Ch group box adjustment

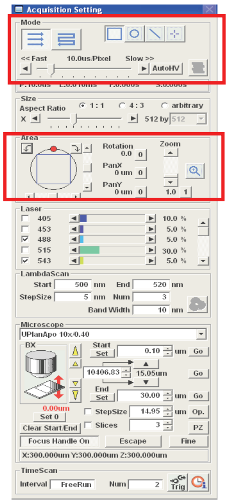
HV: Adjusts detector sensitivity.

Gain: Brightens images with a set magnification.

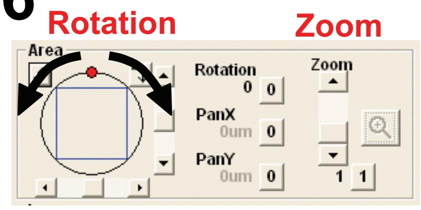
Offset: Darkens the background.

Laser intensity adjustment

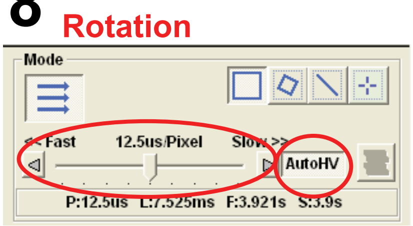

Laser
405
10.0 %
453
5.0 %
488
5.0 %
515
30.0 %
543
5.0 %


Acquisition Setting
Mode
Size
Area
Laser
LambdaScan
Microscope
TimeScan

6


Rotation
Zoom
Area
Rotation
PanX
PanY
Zoom
0
0
0
1

8


Rotation
Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us
L:7.525ms
F:3.921s
S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Click the XY Repeat button XY Repeat to execute a continuous scan.

* If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4.

4. Adjust the focus by turning the focus knob.

5. Make adjustments for green imaging (Alexa488) (laser intensity, HV, offset, etc.).

6. Set the zoom and rotation as required.

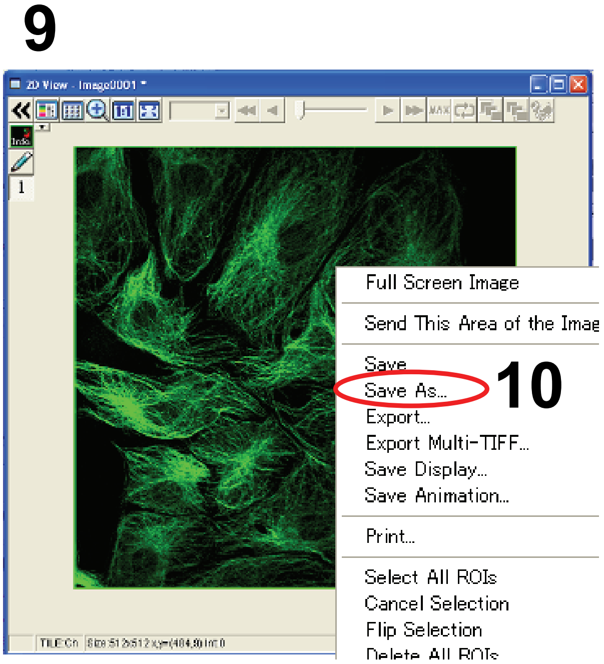
7. After finishing image adjustment, click the STOP button Stop to stop scanning.

8. Select AutoHV and then select ScanSpeed.

* The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
Alternatively, Kalman integration can be used.
For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Single-color staining)

Example: single-color staining with green fluorescence (Alexa488)


9
2D View - Image0001
Full Screen Image
Send This Area of the Imag
Save
Save As...
10
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

9. To acquire the image, click the XY button.

10. Save the image: Select **Save As** by right-clicking or going to the File menu, and then save the image in OIB or OIF format.

■ **Memo** ■
FV10-ASW dedicated file formats

OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an image as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

[Points on acquiring clear images]

For dark images

Increasing the HV: Increases detector sensitivity. Use another method in addition to this if sufficient brightness cannot be obtained even when the HV is increased to 700 V or higher.

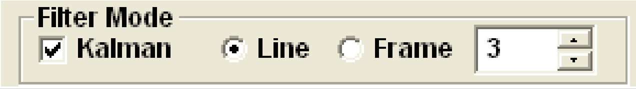
Increasing laser intensity: Increases fluorescent brightness with an increase in excitation light output. Note that an excessively increased intensity may fade fluorescence or cause greater damage to samples.

Increasing the CA: The Auto button stays clicked during regular use so that the optimal pinhole diameter is always automatically set.
Higher brightness can be obtained by deselecting Auto to increase the CA (confocal aperture), which is equivalent to decreasing the Z-axis resolution.

Increasing the gain: Brightens images with the magnification set before image acquisition.

For noisy images

Kalman integration ... Averages images while acquiring as many images as specified. Check the checkbox for Kalman and then select Line (or Frame). Enter **the number of image integrations (the number of scans)**.

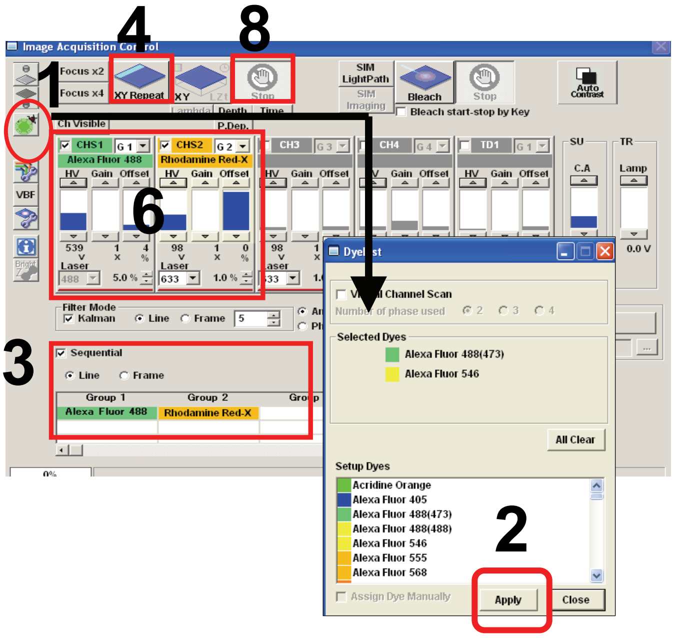


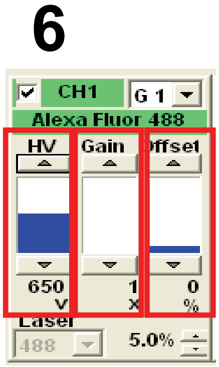
Filter Mode
☑ Kalman ● Line ○ Frame 3

XY Image Acquisition (Multicolor staining)

Line sequential scan

* Mode for avoiding fluorescent crosstalk by individually acquiring fluorescent images


4
8
1
6
3
2
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
Ch Visible
CH1
G 1
Alexa Fluor 488
CH2
G 2
Rhodamine Red-X
CH3
CH4
TD1
HV
Gain
Offset
Laser
488
5.0 %
633
1.0 %
Filter Mode
Kalman
Line
Frame
Sequential
Line
Frame
Group 1
Group 2
Alexa Fluor 488
Rhodamine Red-X
DyeList
Virtual Channel Scan
Number of phase used
Selected Dyes
Alexa Fluor 488(473)
Alexa Fluor 546
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close


6
CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650
V
1
X
0
%
Laser
488
5.0%

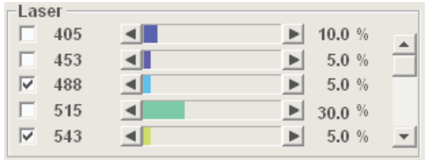
Ch group box adjustment

HV: Adjusts detector sensitivity.

Gain: Brightens images with a set magnification.

Offset: Darkens the background.

Laser intensity adjustment



Laser

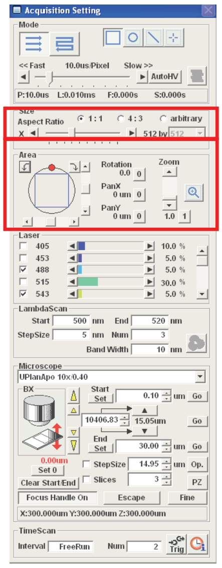
☐ 405 10.0 %

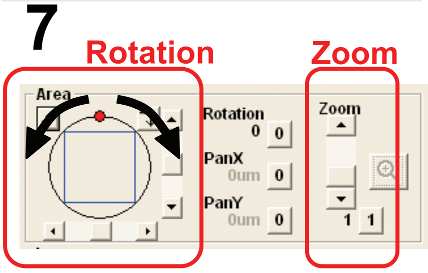
☐ 453 5.0 %

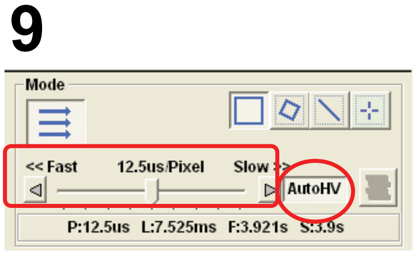
☑ 488 5.0 %

☐ 515 30.0 %

☑ 543 5.0 %


Acquisition Setting
Mode
Size
Aspect Ratio
Area
Rotation
Zoom
Laser
LambdaScan
Microscope
TimeScan


7
Rotation
Zoom
Area
Rotation
0
PanX
0um
0
PanY
0um
0
Zoom
1
1


9
Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us L:7.525ms F:3.921s S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

> Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
> The slit width and positions can be changed manually.
> For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Check the checkbox for Sequential and then select Line.

4. Click the XY Repeat button XY Repeat to execute a continuous scan.

   * If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button Focus x2 Focus x4 .

5. Adjust the focus by turning the focus knob.

6. Make adjustments for CH1/CH2 imaging (laser intensity, HV, offset, etc.).

7. Set the zoom and rotation as required.

8. After finishing image adjustment, click the STOP button Stop to stop scanning.

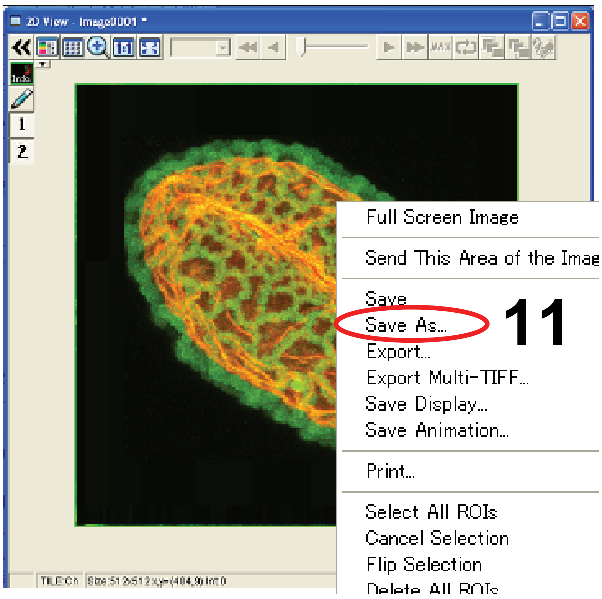
9. Select AutoHV and then select ScanSpeed.

   * The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
   Alternatively, Kalman integration can be used.
   For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Multicolor staining)

Line sequential scan

10


2D View - Image0001
1
2
Full Screen Image
Send This Area of the Imag
Save
Save As...
11
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

10. To acquire the image, click the XY button.
11. Save the image: Select **Save As** by right-clicking or going to the File menu, and then save the image in OIB or OIF format.

■ **Memo** ■
FV10-ASW dedicated file formats

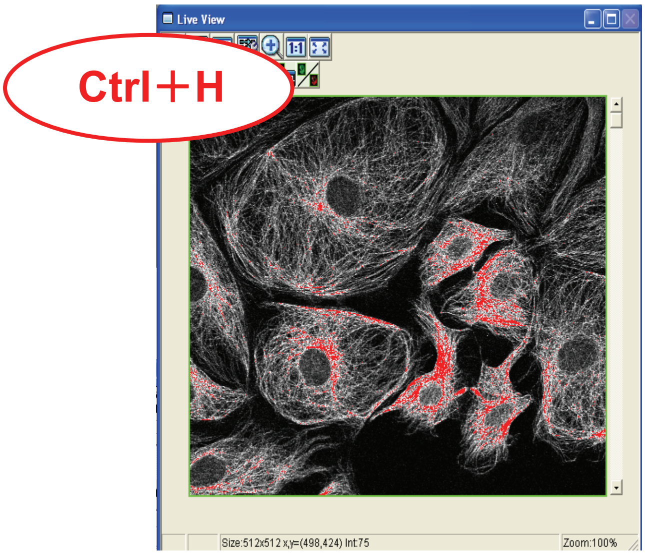
OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

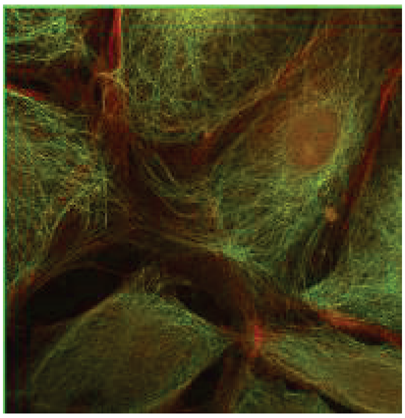
OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an image as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

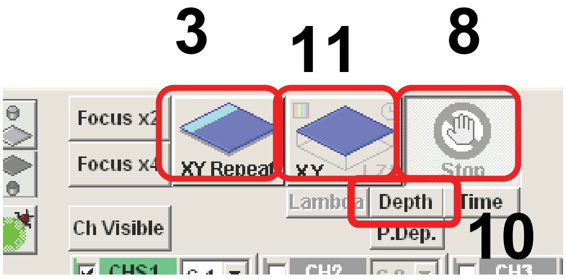
■ Image brightness adjustment ■

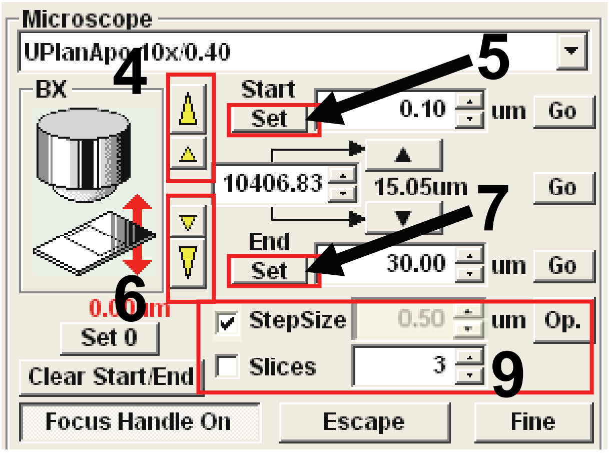
By pressing **Ctrl + H** on the keyboard, an image can be displayed in Hi-Low mode. Pixels of intensity 4095 are shown in red.
To return to normal mode from Hi-Low mode, press **Ctrl + H** again. The image returns to its original color.


Live View
Ctrl＋H
Size:512x512 x,y=(498,424) Int:75
Zoom:100%



XYZ Image Acquisition

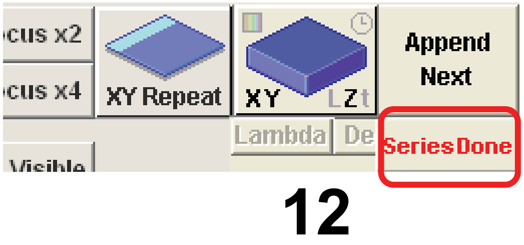

3
11
8
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
P.Dep.
10
Ch Visible
CHS1
CH2
CH3


Microscope
UPlanApo 10x/0.40
BX
4
Start
Set
0.10
um
Go
5
10406.83
15.05um
Go
7
End
Set
30.00
um
Go
6
0.00um
Set 0
StepSize
0.50
um
Op.
Slices
3
9
Clear Start/End
Focus Handle On
Escape
Fine

■ Memo ■

△ : Each click shifts the position by the value entered for StepSize.

△ : Each click shifts the position by 1/2 the value entered for StepSize.


cus x2
cus x4
XY Repeat
XY
LZt
Append
Next
Lambda
De
Series Done
Visible
12

1. Before starting the following procedure, make adjustments for XY imaging. (Refer to page 6 or 8.)

The following procedure fixes the upper and lower limit positions of a continuous cross-sectional image.

2. Click Clear Start/End to clear the settings of acquisition start and end position. Then, click Set 0 to zero the Z-position values being displayed.

3. Click the XY Repeat button to execute a scan.

4. Click △ or △ to shift the focus. (Refer to ■ Memo 1 ■.)

5. When the upper limit position of the sample is displayed in the image, fix the position by clicking the Set button.

6. Click ▽ or ▽ to shift the focus. (Refer to ■ Memo 1 ■.)

7. When the lower limit position of the sample is displayed in the image, fix the position by clicking the Set button.

8. To stop scanning, click the Stop button.

9. Enter a value in StepSize or Slices, and check ☑ the checkbox.

   (Click the Op button to see a recommended value.)

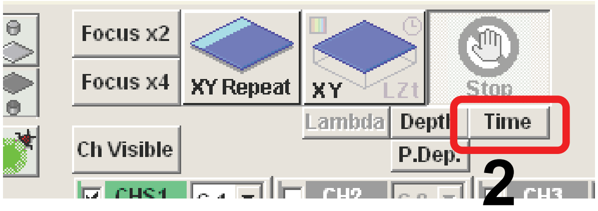
10. Select Depth.

11. To acquire the image, click the XYZ button.

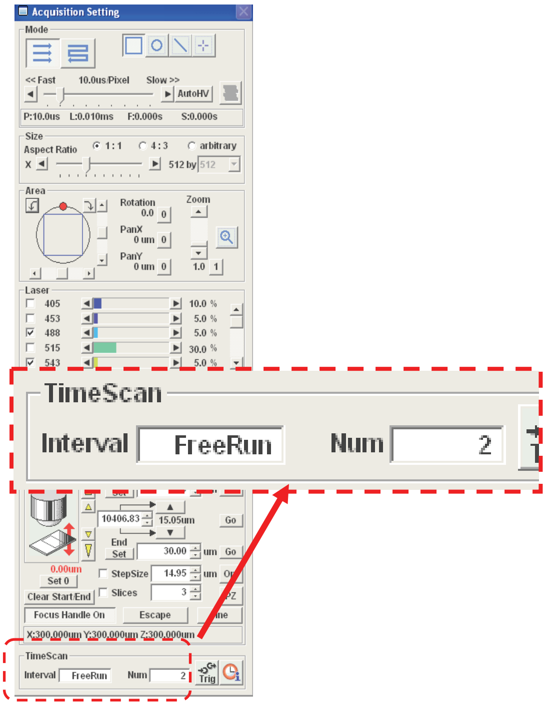
12. To save the image, click Series Done.

For more information on 3D image display, refer to the Supplemental Operations section.

XYT Image Acquisition

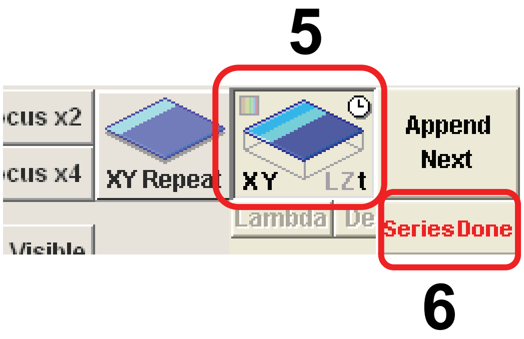

Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
P.Dep.
Ch Visible
CHS1
CH2
CH3
2

1. Before starting the following procedure, make adjustments for XY imaging.
   (Refer to page 6 or 8.)
2. Select Time from among the series buttons.


Acquisition Setting
Mode
Size
Area
Laser
TimeScan

Interval FreeRun Num 2

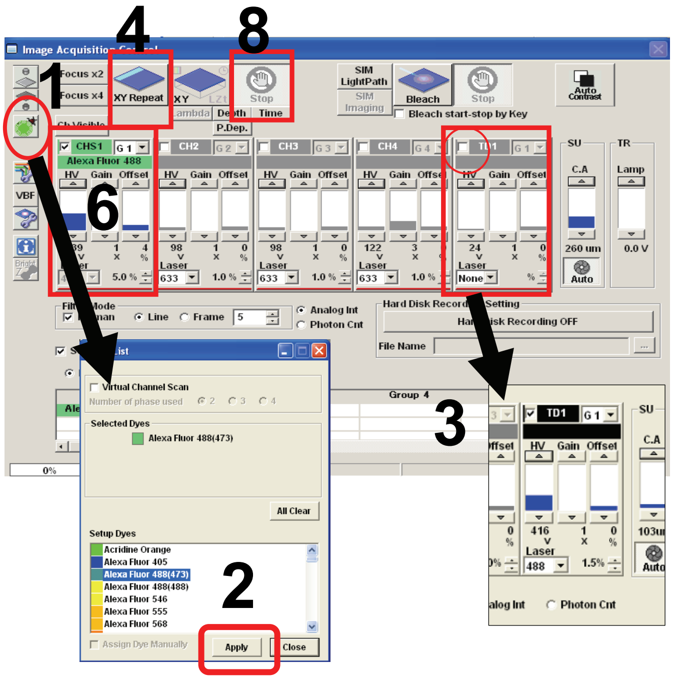
3. In the Interval box, enter the time (in seconds) taken between one acquisition start and the next acquisition start.
   Example: To set a 1 minute interval, type “60” and click Enter.
4. Enter the number of acquisition in the Num box.


5
cus x2
cus x4
XY Repeat
XY
LZt
Append
Next
Lambda
De
Series Done
Visible
6

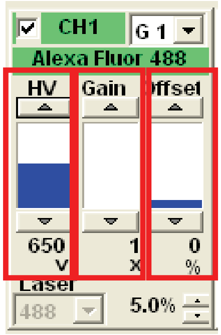
5. To acquire the images, click the XYT button.
6. To save the images, click Series Done.

XY Image Acquisition (Single-color staining + DIC)

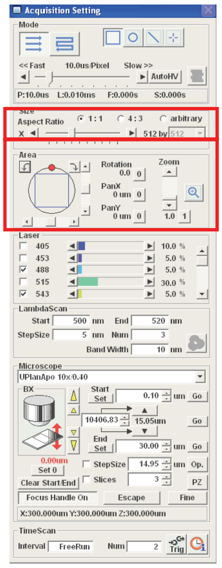
Example: green fluorescent + DIC image


4
8
1
6
3
2
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
P.Dep.
Stop
SIM LightPath
SIM Imaging
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
VBF
CHS1
CH2
CH3
CH4
TD1
Alexa Fluor 488
HV
Gain
Offset
Laser
SU
TR
C.A
Lamp
260 um
0.0 V
Auto
Line
Frame
Analog Int
Photon Cnt
Hard Disk Recording Setting
Hard Disk Recording OFF
File Name
Group 4
Virtual Channel Scan
Number of phase used
Selected Dyes
Alexa Fluor 488(473)
All Clear
Setup Dyes
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488(473)
Alexa Fluor 488(488)
Alexa Fluor 546
Alexa Fluor 555
Alexa Fluor 568
Assign Dye Manually
Apply
Close

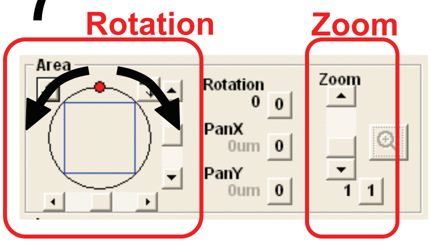
6


CH1
G 1
Alexa Fluor 488
HV
Gain
Offset
650
V
1
X
0
%
Laser
488
5.0%

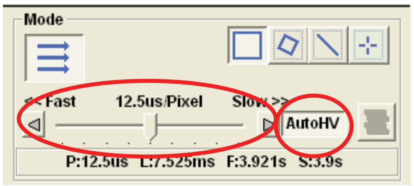
HV:	Adjusts detector sensitivity.
Gain:	Brightens images with a set magnification.
Offset:	Darkens the background.


Acquisition Setting
Mode
Size
Aspect Ratio
Area
Rotation
Zoom
PanX
PanY
Laser
LambdaScan
Microscope
TimeScan

7


Rotation
Zoom
Area
Rotation
PanX
PanY
0um
Zoom

9


Mode
<< Fast
12.5us/Pixel
Slow >>
AutoHV
P:12.5us
L:7.525ms
F:3.921s
S:3.9s

1. From the DyeList panel, select and double-click a fluorescence reagent to observe.

   Suitable slit width, positions, and other settings are determined for the selected fluorescent dye.
   The slit width and positions can be changed manually.
   For more information, refer to the Supplemental Operations section.

2. Click the Apply button.

3. Check the checkbox for TD1.

   Check that the polarizer and the DIC slider for transmitted light are within the optical path limits.

4. Click the XY Repeat button **XY Repeat** to execute a continuous scan.

   * If you are concerned about color fading or damage due to a continuous scan when scanning living cell samples and such, it is recommended that images be shot with decimation by using the FocusX2 or X4 button **Focus x2** **Focus x4**.

5. Adjust the focus by turning the focus knob.

6. Make adjustments for green imaging (Alexa488) and TD imaging.

7. Set the zoom and rotation as required.

8. After finishing image adjustment, click the STOP button **Stop** to stop scanning.

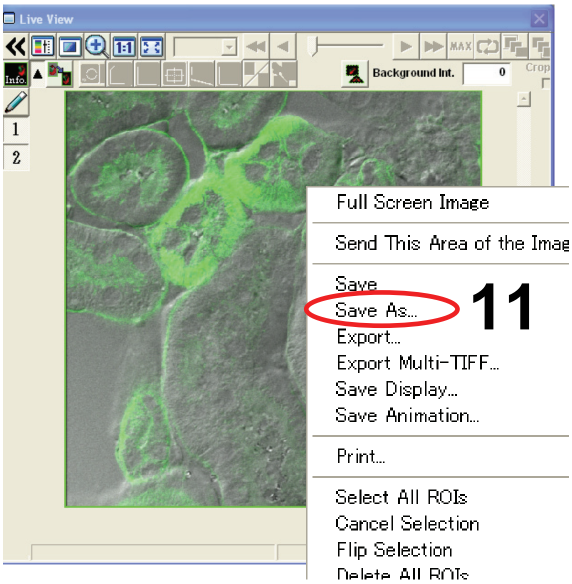
9. Select AutoHV and then select ScanSpeed.

   * The closer the control slider is to Slow, the more noise can be reduced while retaining the current brightness.
   Alternatively, Kalman integration can be used.
   For more information, refer to page 8, “Points on acquiring clear images.”

XY Image Acquisition (Single-color staining + DIC)

Example: green fluorescent + DIC image

10


Live View
Info.
Background Int.
0
MAX
Crop
1
2
Full Screen Image
Send This Area of the Imag
Save
Save As...
11
Export...
Export Multi-TIFF...
Save Display...
Save Animation...
Print...
Select All ROIs
Cancel Selection
Flip Selection
Delete All ROIs

10. To acquire the image, click the XY button.

11. Save the image: Select **Save As** by right-clicking or going to the File menu and then save the image in OIB or OIF format.

■ Memo ■
FV10-ASW dedicated file formats

OIF format:
Two files are created for each OIF file: a file in which the image is saved (16-bit TIFF file) and a sub-file. These two files are required to open the saved file.

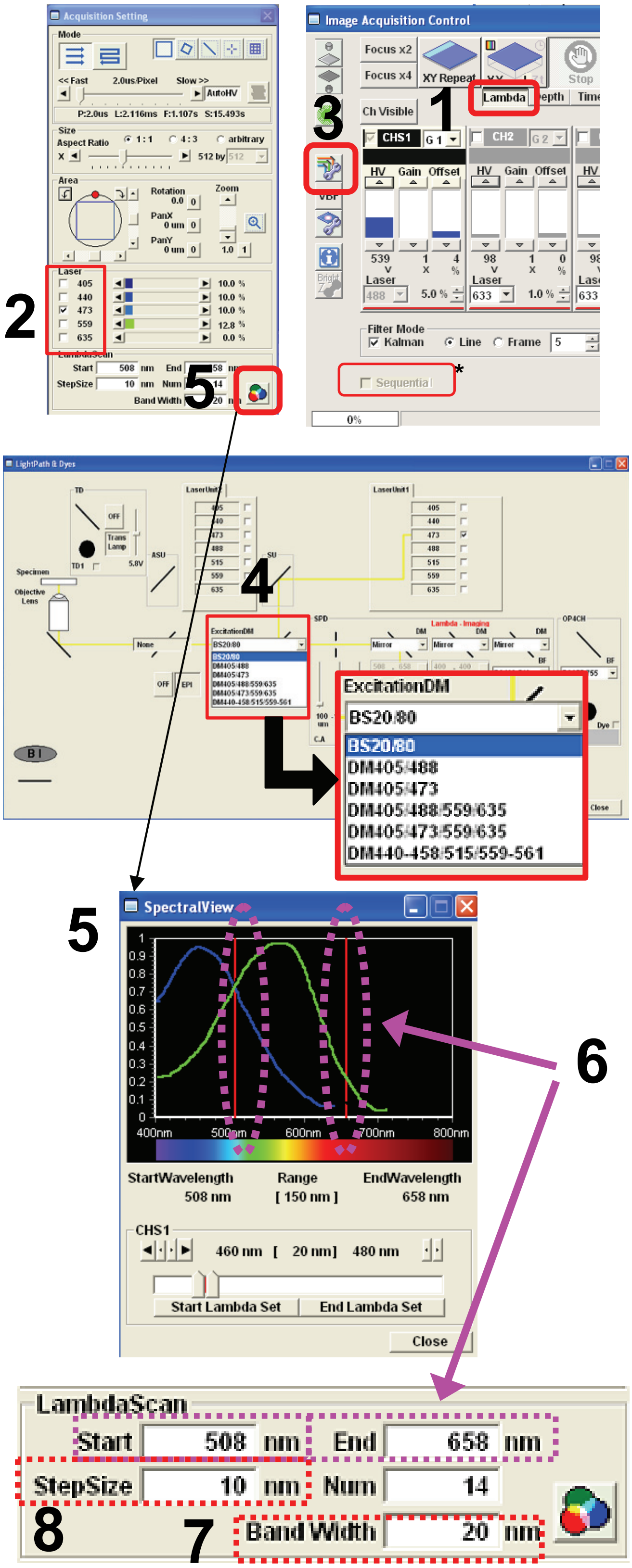
OIB format:
In an OIB file, the two OIF files are combined into one file. The OIB format is more convenient in terms of mobility such as when the file has to be moved.

For more information on how to export an images as a TIFF, JPEG, or other such file, refer to the Supplemental Operations section.

Only for SPD

XYλ Image Acquisition

Spectral image acquisition


Acquisition Setting
Image Acquisition Control
Lambda
Sequential
1
2
3
4
5
*
LightPath & Dyes
ExcitationDM
SpectralView
6
7
8
Start Lambda Set
End Lambda Set
Close

ExcitationDM: BS20/80

- BS20/80
- DM405/488
- DM405/473
- DM405/488/559/635
- DM405/473/559/635
- DM440-458/515/559-561

SpectralView: StartWavelength 508 nm; Range [150 nm]; EndWavelength 658 nm

CHS1: 460 nm [20 nm] 480 nm

LambdaScan: Start 508 nm; End 658 nm; StepSize 10 nm; Num 14; Band Width 20 nm

1. Click **Lambda** .

   * When the Sequential checkbox is checked, the Lambda button is grayed out. Uncheck the checkbox.

2. Check the checkbox for the laser to be used.

3. To display the optical path diagram, click ⚙ .

4. Select BS20/80 or a DM according to the excitation wavelength.

   Example: Select DM405/473 for excitation at 473.

5. To display SpectralView, click ● .

6. In the Start and End boxes, enter the wavelength at which to start and end image acquisition.

   These boxes correspond to the red bars in the SpectralView graph.

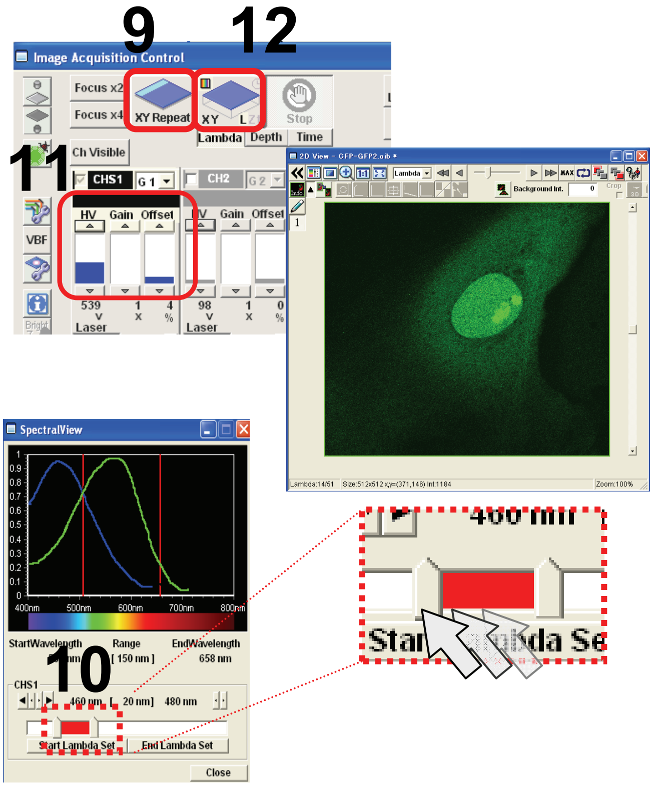
7. In the Band Width box, set the wavelength range of acquisition per frame (Example: 20 nm).

8. In the StepSize box, enter the wavelength interval between one acquisition start and the next acquisition start (Example: 10 nm).

   The minimum settable unit is 1.0 nm.

XYλ Image Acquisition

Spectral image acquisition


9
12
11
10
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
LZ
Stop
Lambda
Depth
Time
Ch Visible
CHS1
G 1
CH2
G 2
HV
Gain
Offset
VBF
539
1
4
98
1
0
Laser
2D View - GFP-GFP2.oib
Background Int.
Lambda:14/51
Zoom:100%
SpectralView
400nm
500nm
600nm
700nm
800nm
StartWavelength
Range
[150 nm]
EndWavelength
658 nm
CHS1
460 nm
[20 nm]
480 nm
Start Lambda Set
End Lambda Set
Close
400 nm
Start Lambda Se

9. To execute a scan, click the XY Repeat button XY Repeat .

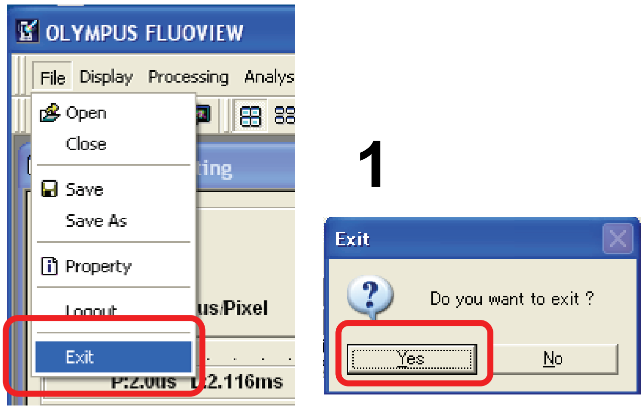
10. While observing the image, find the brightest fluorescent wavelength range by clicking the left slit.

11. Adjust the image.

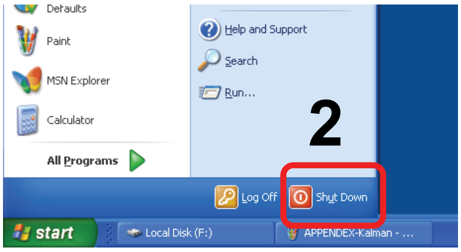
12. To acquire the image, click the XYL button XY LZt .

13. To save the λ image, click SeriesDone SeriesDone .

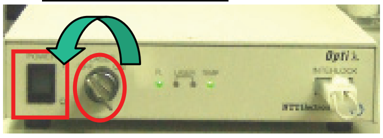
System Shutdown


OLYMPUS FLUOVIEW
File Display Processing Analys
Open
Close
Save
Save As
Property
Logout
Exit
us/Pixel
P:2.0us L:2.116ms
1
Exit
Yes
No

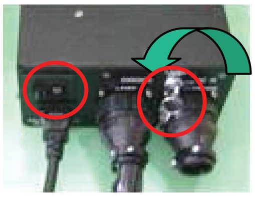
Do you want to exit ?


Defaults
Paint
MSN Explorer
Calculator
All Programs
Help and Support
Search
Run...
2
Log Off
Shut Down
start
Local Disk (F:)
APPENDEX-Kalman - ...

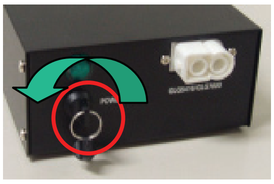
3 **B: LD559nm**

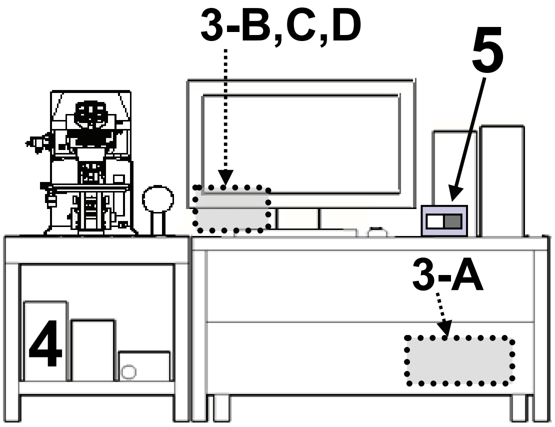


C: MAr laser



D: HeNe (G) laser





1. Exit the FV10-ASW software by selecting Exit from the File menu.
2. Shut down Windows.
3. Turn off the laser systems.

   A: MCPSU (405/440/473/635 nm lasers)
   Switch off the system.

   B: LD559nm
   Turn off the key switch to turn off the main power.

   C: Multi Ar laser
   Turn off the key switch to turn off the main power.

   D: HeNe (G) laser (543 nm)
   Turn off the key switch.
4. Switch off the power of the mercury lamp.
5. Switch off the central power.

Image Acquisition

— Applications —

XYT Image Acquisition

(Photobleaching with a single scanner)

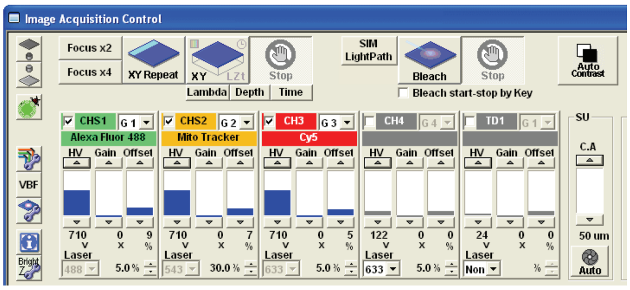
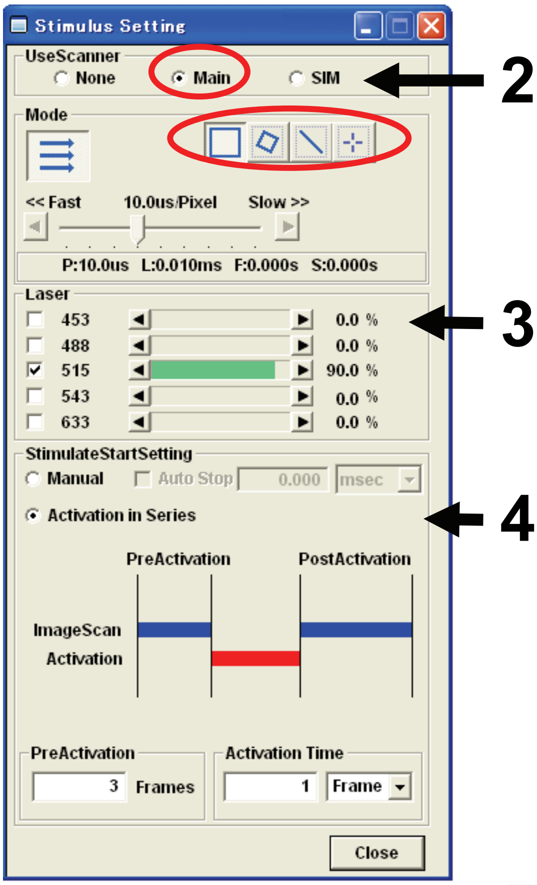


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
CHS1
Alexa Fluor 488
CHS2
Mito Tracker
CH3
Cy5
CH4
TD1
HV
Gain
Offset
Laser
VBF
SU
C.A
50 um
Auto

1. Make imaging adjustments.


Stimulus Setting
Mode
<< Fast
10.0us/Pixel
Slow >>
P:10.0us L:0.010ms F:0.000s S:0.000s
PreActivation
PostActivation
ImageScan
Activation
Close
2
3
4

UseScanner: ○ None ● Main ○ SIM

Laser:

☐	453	0.0 %
☐	488	0.0 %
☑	515	90.0 %
☐	543	0.0 %
☐	633	0.0 %

StimulateStartSetting: ○ Manual ☐ Auto Stop 0.000 msec

● Activation in Series

PreActivation: 3 Frames

Activation Time: 1 Frame

2. Select Main in the Use Scanner window. Enclose the region to be photobleached using controls in the Mode section.

3. In the Laser section, select the photobleaching power.

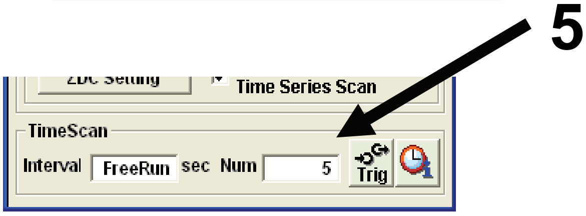
4. Set StimulateStartSetting.

When linking photobleaching with imaging

  4-1. Click the radio button for Activation in Series.

  4-2. Enter the PreActivation and Activation Time fields.

(For manual operation, refer to the following page.)

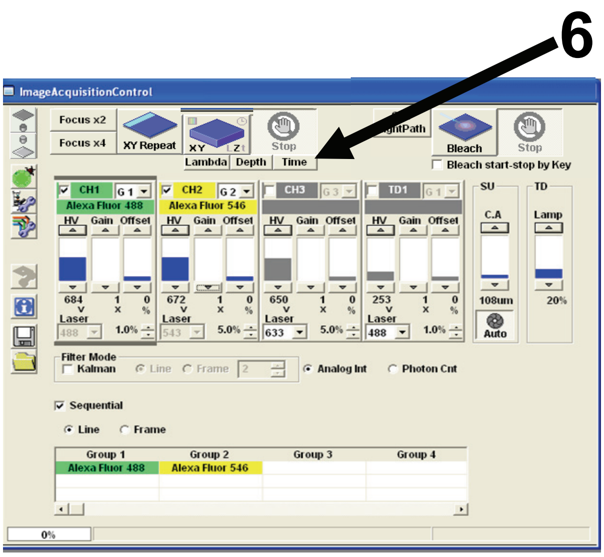

ZDC Setting
Time Series Scan
Trig
5

TimeScan: Interval FreeRun sec Num 5

5. Enter the time interval and number.

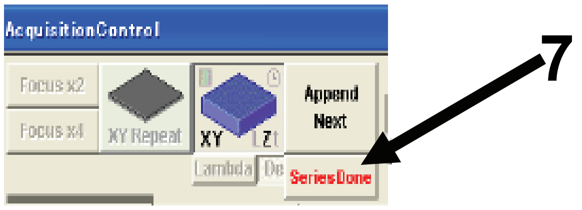
XYT Image Acquisition

(Photobleaching with a single scanner)


6
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
Bleach
Stop
Bleach start-stop by Key
CH1
Alexa Fluor 488
CH2
Alexa Fluor 546
CH3
TD1
Filter Mode
Kalman
Sequential
Group 1
Group 2
Group 3
Group 4

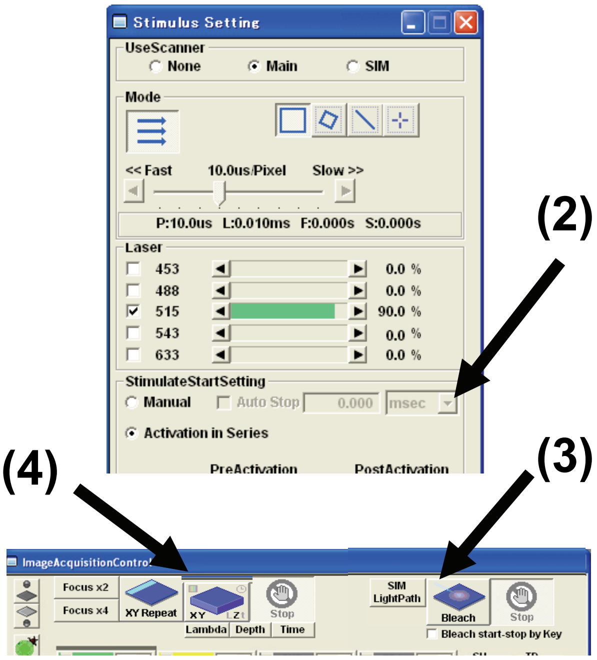
6. With Time selected, click the XYT button to execute image acquisition.

When linking photobleaching with imaging
The region is automatically photobleached at a set time.


7
AcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Append
Next
SeriesDone

7. Clicking SeriesDone causes the window bar of the acquired image to show “2DView-LiveImage(x).”

8. Save the image:
Save the image by selecting Save As from File on the menu bar.
(The Save As types “oib” and “oif” are the dedicated file formats for the FV10-ASW software.)


Stimulus Setting
UseScanner
None
Main
SIM
Mode
<< Fast
10.0us/Pixel
Slow >>
P:10.0us L:0.010ms F:0.000s S:0.000s
Laser
453
488
515
543
633
90.0 %
StimulateStartSetting
Manual
Auto Stop
msec
Activation in Series
PreActivation
PostActivation
(2)
(3)
(4)
ImageAcquisitionControl
XY Repeat
XY
Bleach
Stop
SIM LightPath
Bleach start-stop by Key

When photobleaching manually

(1) Perform operations 1 to 3 on the previous page.

(2) Click the radio button for Manual.
(To set the amount of time the Bleach button is enabled when it is clicked, check the checkbox for Auto Stop and enter the number of seconds.)

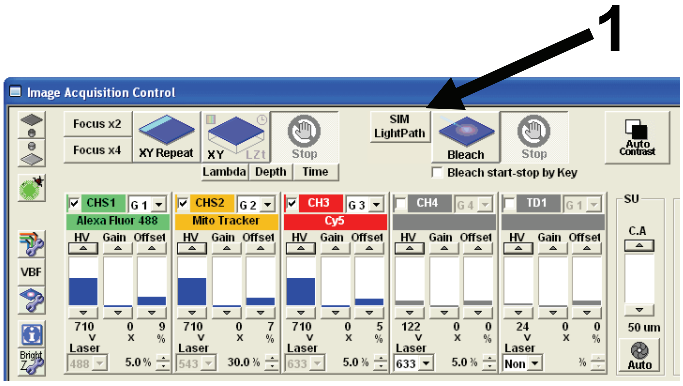
(3) Click the Bleach button.

(4) Observe the image in XY Repeat, XY, or XYT.

Only for SIM

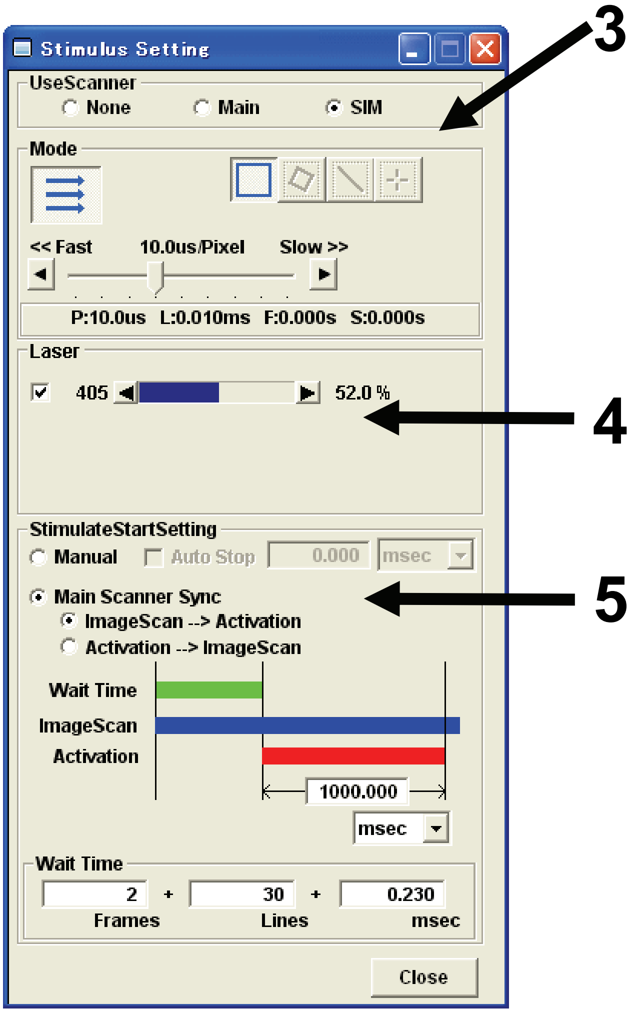
XYT Image Acquisition

(Photobleaching with a SIM scanner)


1
Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
LZt
Stop
Lambda
Depth
Time
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
Auto Contrast
VBF
CHS1 G1 Alexa Fluor 488
CHS2 G2 Mito Tracker
CH3 G3 Cy5
CH4 G4
TD1 G1
HV Gain Offset
710 0 9
710 0 7
710 0 5
122 0 0
24 0 0
Laser
488 5.0 %
543 30.0 %
633 5.0 %
633 5.0 %
Non
SU
C.A
50 um
Auto

1. Click SIM LightPath.

2. Make imaging adjustments.


3
4
5
Stimulus Setting
Close

UseScanner
○ None ○ Main ● SIM

Mode

<< Fast 10.0us/Pixel Slow >>

P:10.0us L:0.010ms F:0.000s S:0.000s

Laser

☑ 405 52.0 %

StimulateStartSetting

○ Manual ☐ Auto Stop 0.000 msec

● Main Scanner Sync

● ImageScan --> Activation

○ Activation --> ImageScan

Wait Time
ImageScan
Activation
1000.000 msec

Wait Time
2 Frames + 30 Lines + 0.230 msec

3. Select SIM in the Use Scanner window. Enclose the region to be photobleached using controls in the Mode section.

4. In the Laser section, select the photobleaching power.

5. Set StimulateStartSetting.

When photobleaching manually

5-1. Click the radio button for Manual.

(To set the amount of time the Bleach button is enabled when it is clicked, check the checkbox for Auto Stop and enter the number of seconds.)

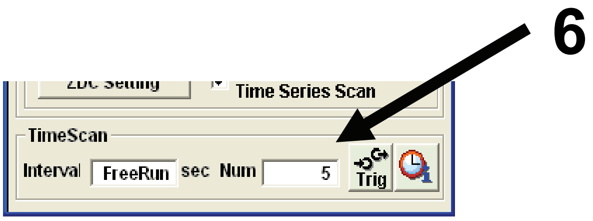
When linking photobleaching with imaging

5-1. Click the radio button for Main Scanner Sync and for ImageScan → Activation.

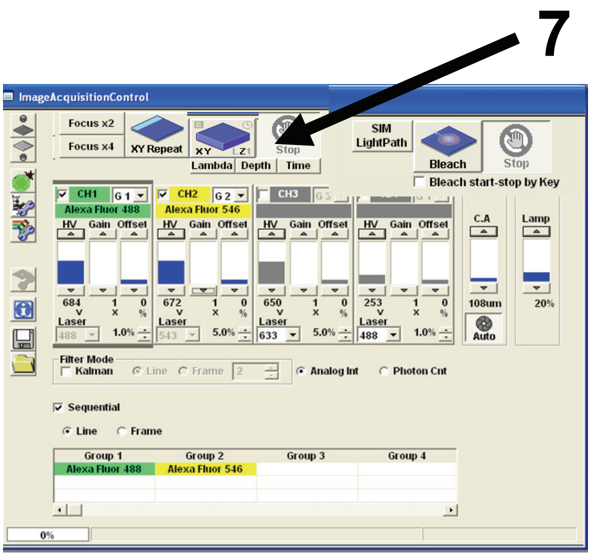
5-2. Enter the values for Activation and Wait Time.

XYT Image Acquisition

(Photobleaching with a SIM scanner)


6
ZDC Setting
Time Series Scan
TimeScan
Interval
FreeRun
sec
Num
5
Trig

6. Enter the time interval and number.


7
ImageAcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Lambda
Depth
Time
Stop
SIM LightPath
Bleach
Stop
Bleach start-stop by Key
CH1
Alexa Fluor 488
CH2
Alexa Fluor 546
CH3
HV
Gain
Offset
Laser
C.A
Lamp
Auto
Filter Mode
Kalman
Line
Frame
Analog Int
Photon Cnt
Sequential
Line
Frame
Group 1
Group 2
Group 3
Group 4
0%

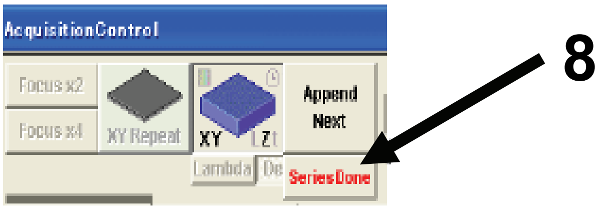
7. With Time selected, click the XYT button to execute image acquisition.

   When photobleaching manually

   Click the Bleach button when you want to start photobleaching.

   When linking photobleaching with imaging

   The region is automatically photobleached at the set time.


8
AcquisitionControl
Focus x2
Focus x4
XY Repeat
XY
Zt
Lambda
De
Append
Next
SeriesDone

8. Clicking SeriesDone causes the window bar of the acquired image to show “2DView-LiveImage(x).”

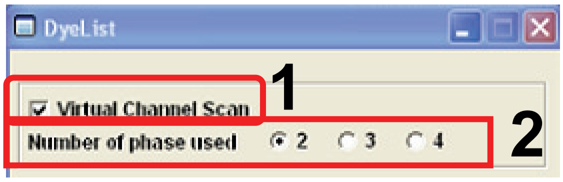
9. Save the image:
   Save the image by selecting Save As from File on the menu bar.
   (The Save As types “oib” and “oif” are the dedicated file formats for the FV10-ASW software.)

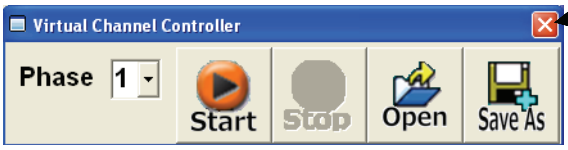
XY Image Acquisition

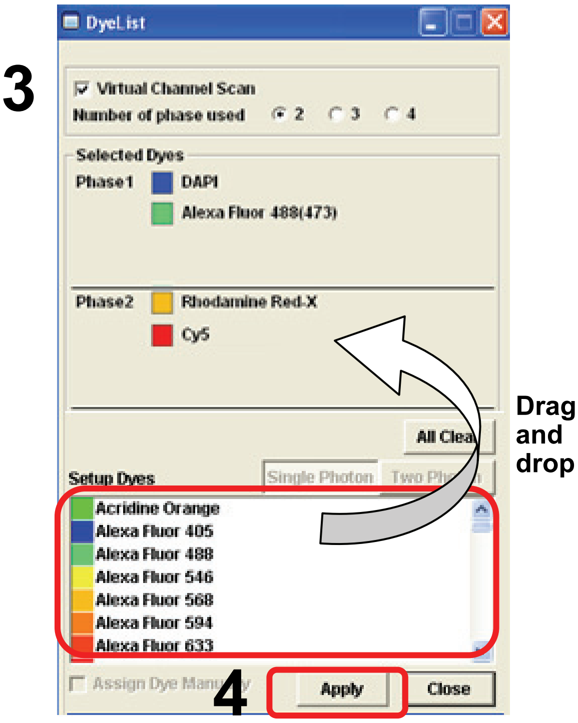
(Four-color staining with virtual channel)

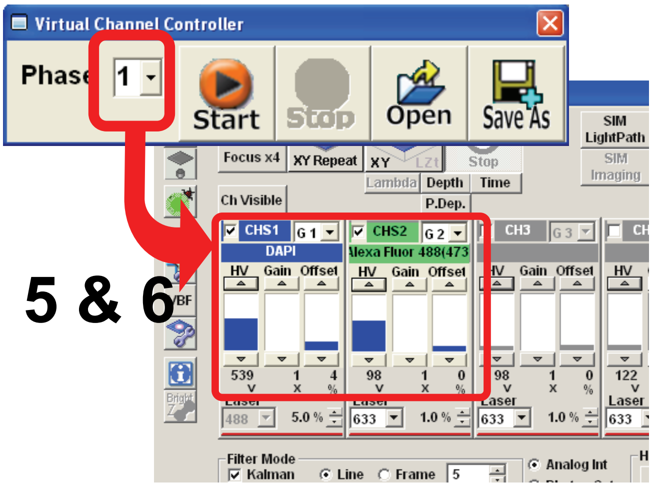
Acquiring a four-color staining image with virtual channel scan

Example: DAPI and Alexa488 for phase 1 / RodaminRed and Cy5 for phase 2


DyeList
☑ Virtual Channel Scan
Number of phase used ● 2 ○ 3 ○ 4
1
2


Virtual Channel Controller
Phase 1
Start
Stop
Open
Save As


3
DyeList
☑ Virtual Channel Scan
Number of phase used ● 2 ○ 3 ○ 4
Selected Dyes
Phase1 DAPI
Alexa Fluor 488(473)
Phase2 Rhodamine Red-X
Cy5
All Clear
Drag and drop
Setup Dyes
Single Photon
Two Photon
Acridine Orange
Alexa Fluor 405
Alexa Fluor 488
Alexa Fluor 546
Alexa Fluor 568
Alexa Fluor 594
Alexa Fluor 633
☐ Assign Dye Manually
4
Apply
Close


Virtual Channel Controller
Phase 1
Start
Stop
Open
Save As
5 & 6
Focus x4
XY Repeat
XY
Lambda
Depth
Time
SIM LightPath
SIM Imaging
Ch Visible
CHS1
DAPI
CHS2
Alexa Fluor 488(473)
CH3
HV
Gain
Offset
Laser
Filter Mode
Kalman
Line
Frame
Analog Int

1. Check the checkbox for ☑ Virtual Channel Scan in DyeList to automatically open the Virtual Channel Controller.

2. In “Number of phase used,” select “2” as the phase count.

3. Drag a dye from the dye list to each Phase.

4. To set the fluorescent dyes and the phase count, click Apply.

5. Select Phase1 to display DAPI and Alexa488 in Image Acquisition Control.

   *** The slit width, filter, and DM settings are switched to DAPI and Alexa488.**

   Adjust the image.

6. Select Phase2 and adjust the image again.

7. To acquire the image, click Virtual Channel Controller Start .

8. Save the image.

The use of this function does not reflect the LUT setting.

After image acquisition finishes, change the LUT setting by using LUT Setting .

HDRi Image Acquisition

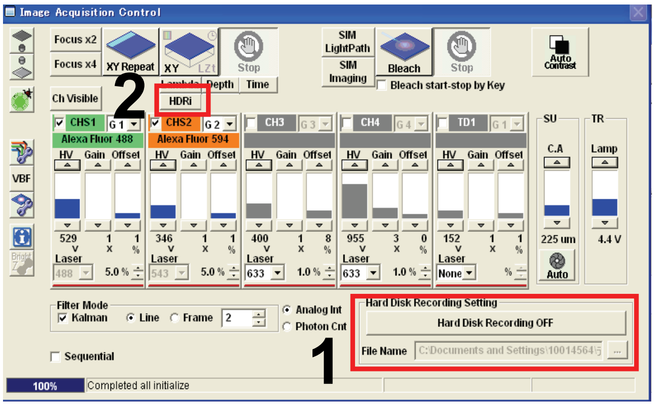


Image Acquisition Control
Focus x2
Focus x4
XY Repeat
XY
Stop
SIM LightPath
SIM Imaging
Bleach
Stop
Auto Contrast
Ch Visible
HDRi
Lambda
Depth
Time
Bleach start-stop by Key
2
CHS1
G1
Alexa Fluor 488
CHS2
G2
Alexa Fluor 594
CH3
G3
CH4
G4
TD1
G1
HV
Gain
Offset
SU
C.A
TR
Lamp
VBF
529
346
400
955
152
225 um
4.4 V
Laser
488
543
633
633
None
5.0 %
5.0 %
1.0 %
1.0 %
Auto
Filter Mode
Kalman
Line
Frame
2
Analog Int
Photon Cnt
Sequential
1
Hard Disk Recording Setting
Hard Disk Recording OFF
File Name
C:\Documents and Settings\100145645
100%
Completed all initialize

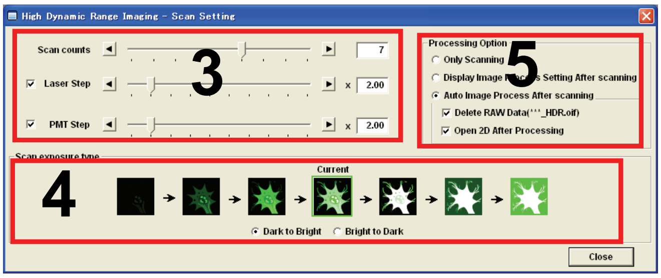
1. After finishing image adjustment, set the save destination for hard disk recording.

2. Turn on **HDRi** in the Image Acquisition Control to open the HDRI Scan Setting window.

3. Set the condition for HDRi.
   - Scan count: number of scans (2 to 10)
   - Laser Step: increase/decrease rate of laser output
   - PMT Step: increase/decrease rate of HV

   *** The value of laser output in Acquisition Setting and the value of HV in Image Acquisition Control are applied to the basic values for HDRi.**

4. Select "Dark to Bright" or "Bright to Dark" and click the icon of acquisition sequence which is given the basic values for HDRi. "Current" is shown on the top of the selected icon.


High Dynamic Range Imaging - Scan Setting
Scan counts
7
3
Laser Step
x 2.00
PMT Step
x 2.00
Processing Option
Only Scanning
Display Image Process Setting After scanning
5
Auto Image Process After scanning
Delete RAW Data(***_HDR.oif)
Open 2D After Processing
Scan exposure type
4
Current
Dark to Bright
Bright to Dark
Close

HDRI Scan Setting window

Example of settings

<Basic values>

Laser output in Acquisition Setting: 10.0 (%)

HV in Image Acquisition Control: 500 (V)

<Condition settings for HDRi >

Scan count = 5, Laser Step = x1.2, PMT Step = x1.2, Dark to Bright, "Current" icon = 3rd from the left

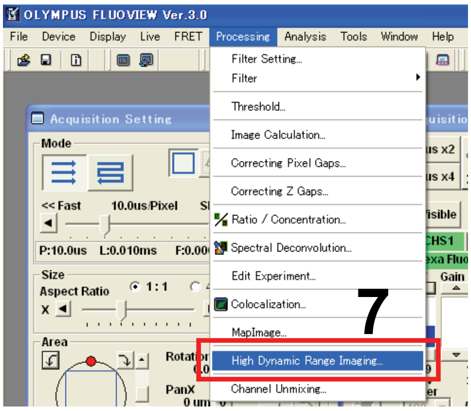
<Setting results>

Laser output (%) : 6.9 -> 8.3 -> 10.0 -> 12.0 -> 14.4

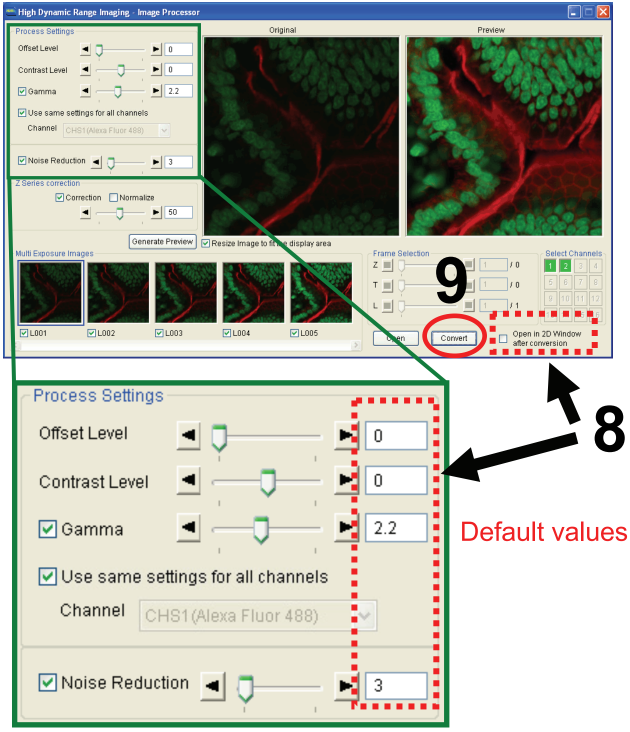
HV (V) : 347 -> 417 -> 500 -> 600 -> 720

5. In the Processing Option section, set the process to be executed after image acquisition finishes.
   - **Only Scanning**: Only executes scanning.
   - **Display Image Process Setting After scanning**: Displays the HDRi Image Processor window after scanning finishes.
   - **Auto Image Process After scanning**: Executes HDRi image processing automatically after scanning finishes. (The parameters used when HDRi Image Processor was last used are applied.)

HDRi Image Acquisition


OLYMPUS FLUOVIEW Ver.3.0
File
Device
Display
Live
FRET
Processing
Analysis
Tools
Window
Help
Acquisition Setting
Mode
Filter Setting...
Filter
Threshold...
Image Calculation...
Correcting Pixel Gaps...
Correcting Z Gaps...
Ratio / Concentration...
Spectral Deconvolution...
Edit Experiment...
Colocalization...
MapImage...
High Dynamic Range Imaging...
Channel Unmixing...
7

HDRi Image Processor window


High Dynamic Range Imaging - Image Processor
Original
Preview
Z Series correction
Correction
Normalize
Generate Preview
Resize image to fit the display area
Multi Exposure Images
L001
L002
L003
L004
L005
Frame Selection
Select Channels
Open
Convert
Open in 2D Window after conversion
9
8
Default values

Process Settings

Offset Level 0

Contrast Level 0

☑ Gamma 2.2

☑ Use same settings for all channels

Channel CHS1(Alexa Fluor 488)

☑ Noise Reduction 3

Offset Level: The intensity less then Offset value is regarded as 0. The low intensity noise on the image can be eliminated.

Contrast Level: The grayscale of entire images can be adjusted.

Gamma: Gamma correction.

Use same settings for all channels: If it is ticked, the same settings are performed in the image processing of all channels.

Noise Reduction: The noise can be reduced without losing the characteristics of the sample.

6. To start scanning images, click the XY button. Scanned images are saved in the set save destination for hard disk recording.

   (File names are assigned with the format ***_**HDR.oif**.)

When Auto Image Process After scanning is selected in step 6, images are saved after being automatically formatted in HDRI (Files named ***_.oif).

7. From the menu, select Processing and then High Dynamic Range Image to open the HDRi Image Processor window.

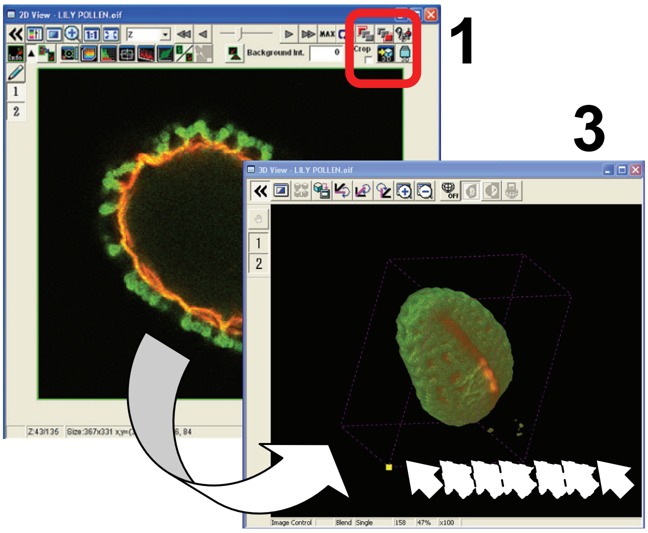
When Display Image Process Setting After scanning is selected in step 6, the window automatically opens.

8. Set the Offset Level, Contrast Level, Gamma and Noise Reduction, and then check the checkbox of “Open in 2D Window after conversion”.

9. To create HDRi images, click the Convert button.

— Supplemental Operations —

3D Image Analysis 3D image creation/cross-sectional observation

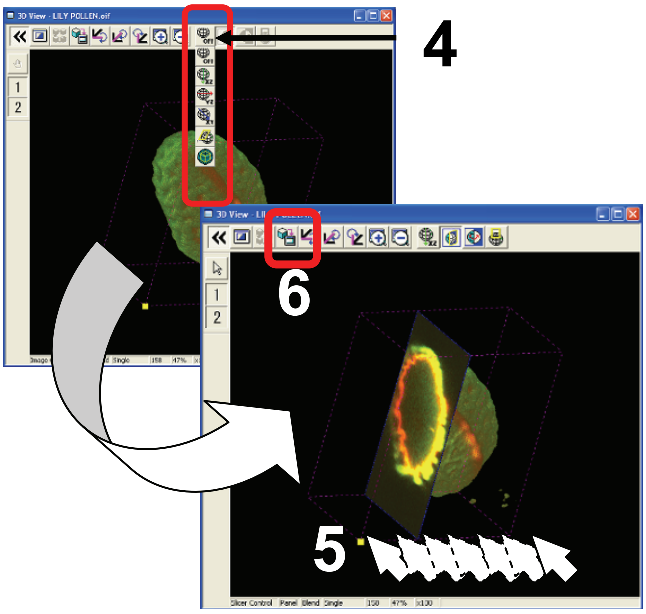

2D View - LILY POLLEN.oif
3D View - LILY POLLEN.oif
1
3

Observe an image from a desired angle.

1. Click 3D of the 2D View-(file name) image.

2. A 3D View is created.

3. To observe the object from a desired angle, move the 3D object by dragging the mouse on the image.

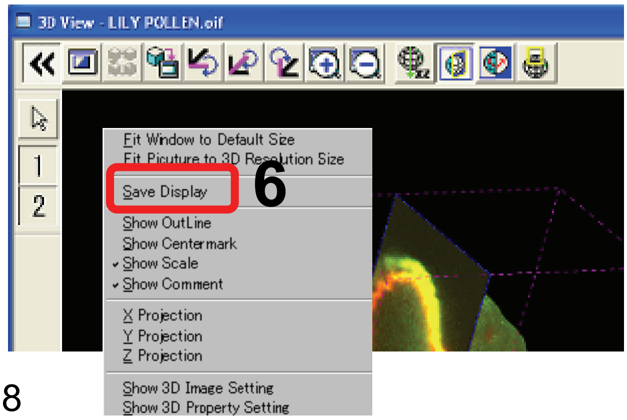

3D View - LILY POLLEN.oif
4
6
5

Observe the desired cross-section of an image.

4. Click OFF and then select XZ.

5. To observe the desired vertical cross-section, move the object right and left by dragging the mouse on the image.

Save the 3D image.


3D View - LILY POLLEN.oif
Fit Window to Default Size
Fit Picuture to 3D Resolution Size
Save Display
6
Show OutLine
Show Centermark
Show Scale
Show Comment
X Projection
Y Projection
Z Projection
Show 3D Image Setting
Show 3D Property Setting

6. Click ▣ to create a 2D image. Save the image by right-clicking.

(Or, right click on the 3D image in order to select Save Display to save the view, including the ROI being displayed, in BMP format.)

3D Image Analysis Detailed settings for 3D image creation

Cut View button
Projects a cross-sectional view on a slice when the slice graphics mode is set.

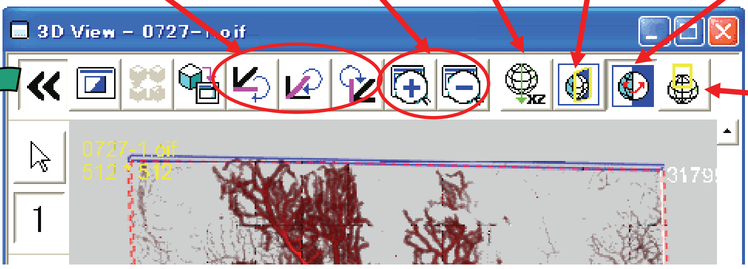
Image zoom in/out

3D image rotation

Slice button

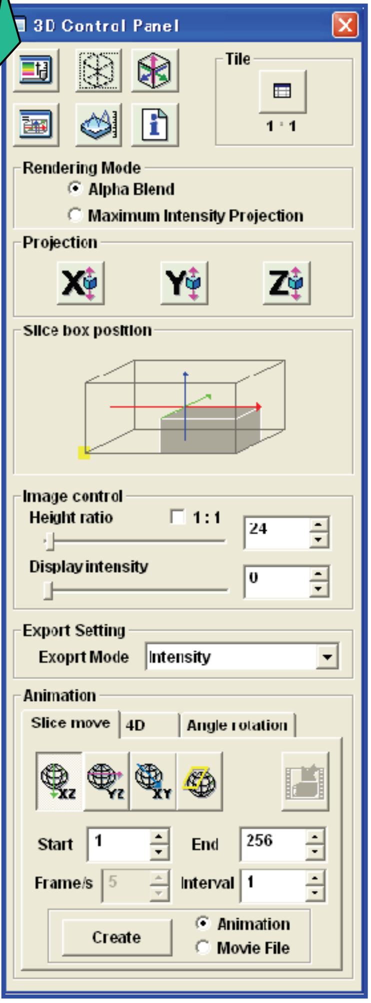
Change View Region button
Switches between the sliced 3D image view region and the hidden region.

Orthogonal View button
Allows for observation with the view point fixed at the front of a sliced cross-section.



3D View – 0727-1.oif

0727-1.oif
512 * 512



3D Control Panel

Tile
1 : 1

Rendering Mode
● Alpha Blend
○ Maximum Intensity Projection

Projection
X
Y
Z

Slice box position

Image control
Height ratio ☐ 1 : 1 24
Display intensity 0

Export Setting
Exoprt Mode Intensity

Animation
Slice move
4D
Angle rotation
XZ
YZ
XY

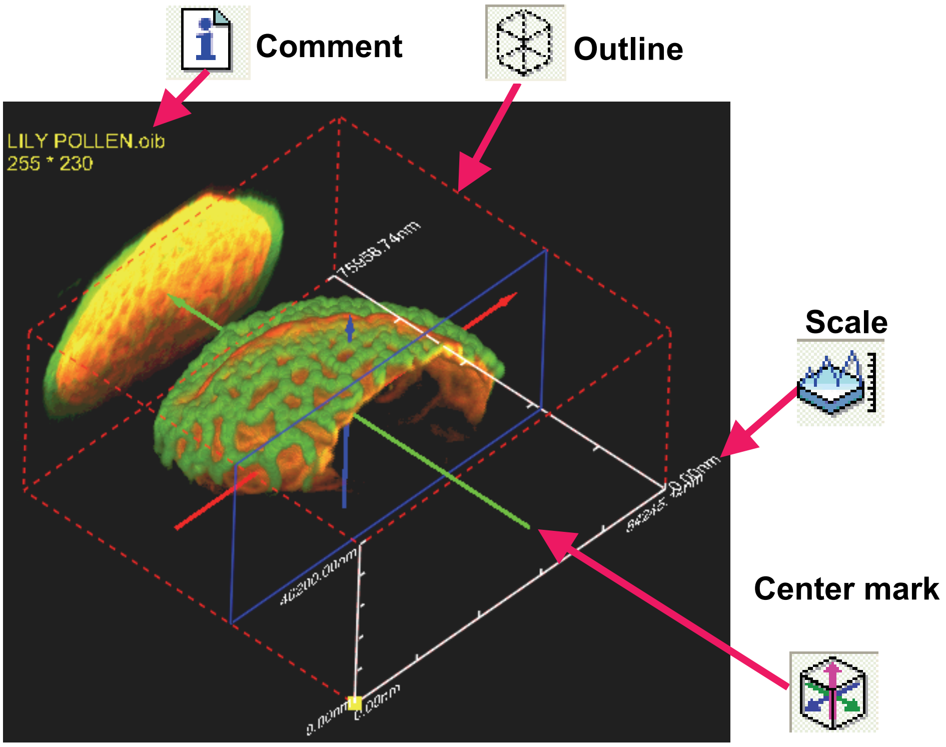
Start 1 End 256
Frame/s 5 Interval 1

Create
● Animation
○ Movie File

3D Image Settings (LUT adjustment, image adjustment, etc.)

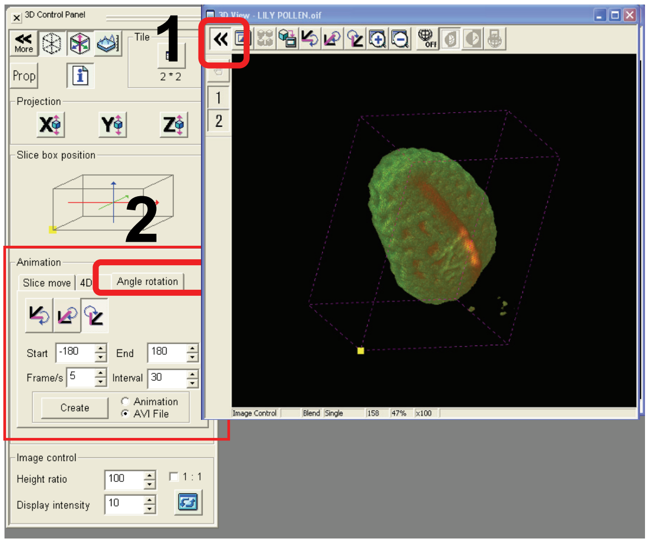
3D Property Settings (image graphics setting such as scale bars and comments)

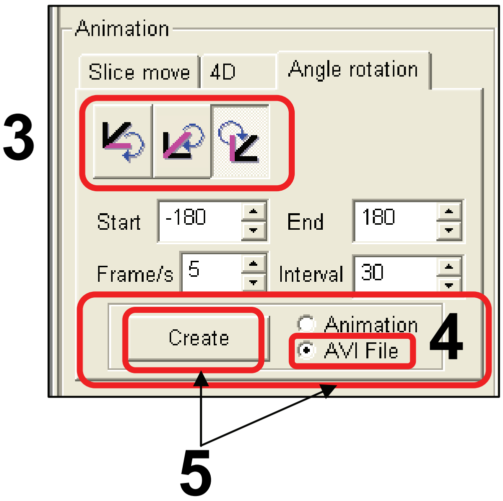
Graphics ON/OFF

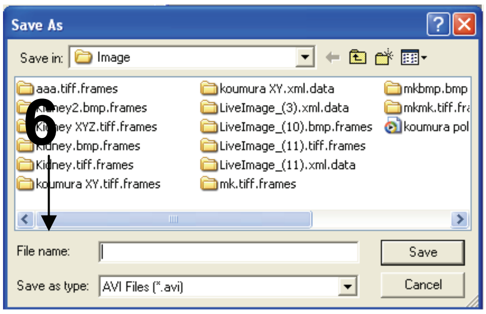


Comment
Outline
Scale
Center mark
LILY POLLEN.oib
255 * 230
75958.74nm
40200.00nm
0.00nm
0.00nm
0.00nm

3D Image Analysis How to create a file

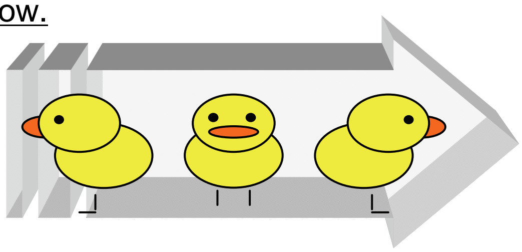

3D Control Panel
More
Tile
2 * 2
Prop
Projection
Slice box position
Animation
Slice move
4D
Angle rotation
Start -180
End 180
Frame/s 5
Interval 30
Create
Animation
AVI File
Image control
Height ratio 100
1 : 1
Display intensity 10
3D View - LILY POLLEN.oif
1
2


3
4
5
Animation
Slice move
4D
Angle rotation
Start -180
End 180
Frame/s 5
Interval 30
Create
○ Animation
● AVI File


6
Save As
Save in: Image
aaa.tiff.frames
kidney2.bmp.frames
kidney XYZ.tiff.frames
kidney.bmp.frames
Kidney.tiff.frames
koumura XY.tiff.frames
koumura XY.xml.data
LiveImage_(3).xml.data
LiveImage_(10).bmp.frames
LiveImage_(11).tiff.frames
LiveImage_(11).xml.data
mk.tiff.frames
mkbmp.bmp
mkmk.tiff.fra
koumura pol
File name:
Save as type: AVI Files (*.avi)
Save
Cancel

To save a rotation file as a movie, render the image in 3D with the following procedure.

As an example, an image is rotated 180º below.



1. Click the « button.

2. Click the Angle rotation tab.

3. Select the rotation axis.

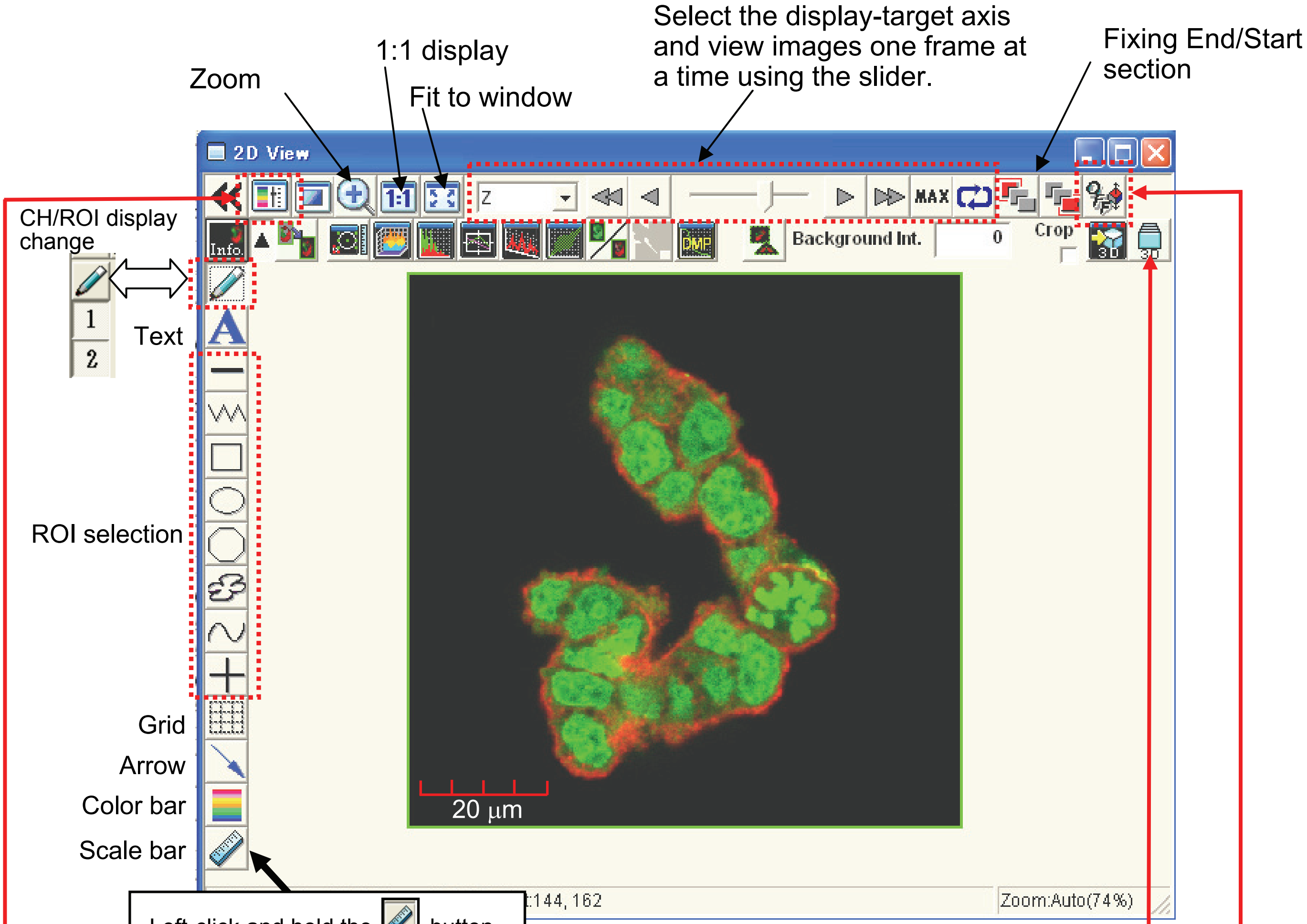
4. Enter the rotation angle.

Start: starting angle / End: ending angle
Frame/s: rotation speed / Interval: angle interval

5. Select AVI File and then click Create.

6. Enter a file name and then click Save.

2D View Window

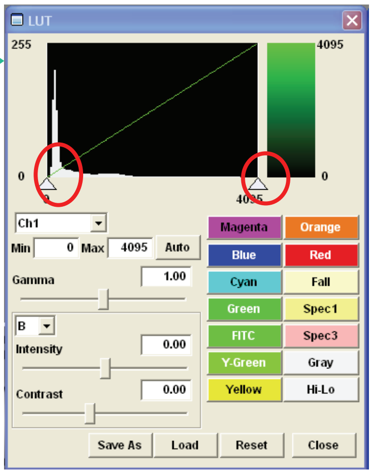

Zoom
1:1 display
Fit to window
Select the display-target axis and view images one frame at a time using the slider.
Fixing End/Start section
CH/ROI display change
Text
ROI selection
Grid
Arrow
Color bar
Scale bar
2D View
Z
MAX
Info.
Crop
1
2
3D
20 µm
:144, 162

Background Int. 0

Zoom:Auto(74%)

Left-click and hold the button to drag it to a place on the image and drop it there.
(A straight line can be drawn by pressing Shift when left-clicking.)

LUT setting (edits image brightness)


LUT
255
4095
0
0
4095
Ch1
Auto
B
Magenta
Orange
Blue
Red
Cyan
Fall
Green
Spec1
FITC
Spec3
Y-Green
Gray
Yellow
Hi-Lo
Save As
Load
Reset
Close

Min 0 Max 4095

Gamma 1.00

Intensity 0.00

Contrast 0.00

Contrast can be freely set by moving the cursor right or left, or by entering values in Min and Max.

Creation of rotating 3D animation

Creates a rotating 3D animation based on the X axis (standard animation).

Creates a rotating 3D animation based on the Y axis (standard animation)

Displays the 3D Animation window that can be used to customize the settings of the rotating 3D animation creation process.
Enter settings and click New Image to create a 3D animation.

Projection mode change

Projection Off button: No projection displayed.

Z Intensity Projection button: Displays Z Intensity Projection images.

T Intensity Projection button: Displays T Intensity Projection images.

λ Intensity Projection button: Displays λ Intensity Projection images.

TOPO Z Topographic Projection button: Displays Z Topographic Projection images.

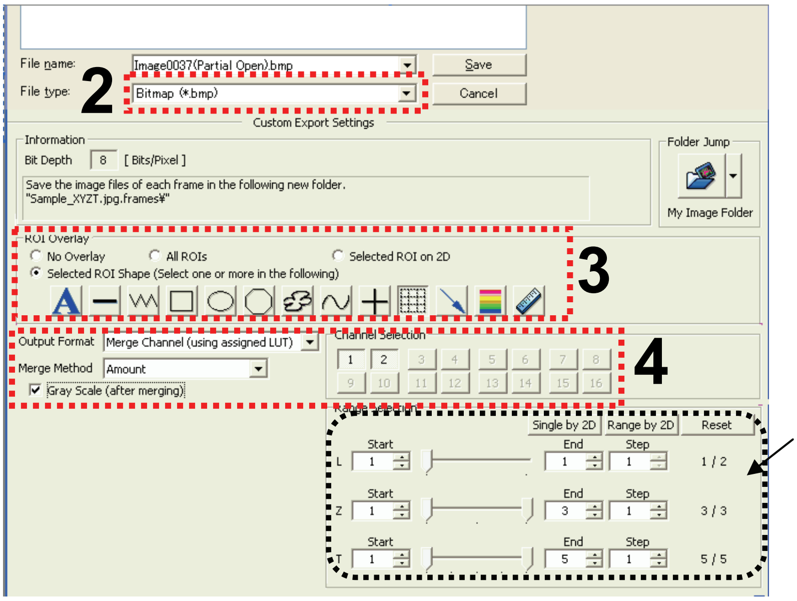
TOPO λ Topographic Projection button: Displays λ Topographic Projection images.

T Series Average button: Displays T series average images.

Acquired Image Export

Saving images as Bitmap (*.bmp), JPEG (*.jpg, *.jpeg), TIFF (*.tif, *.tiff), or PNG (*.png)

1. Open the Live View window or 2D View window of the image to be saved. Right-click on the image and then select Export.
2. Select a file format to use for the export.
3. When saving a ROI in an image, such as a scale bar, select All ROIs or Selected ROI Shape.
4. Select the output mode of the image. In Channel Selection, select an output channel.
    - Merging images
    - LUT images for each CH
    - Raw data (black and white) images
5. To save the file, click Save.


2
3
4
Save
Cancel
Custom Export Settings
Information
Folder Jump
My Image Folder
ROI Overlay
○ No Overlay
○ All ROIs
○ Selected ROI on 2D
Channel Selection
1 2 3 4 5 6 7 8
9 10 11 12 13 14 15 16
Range selection
Single by 2D
Range by 2D
Reset

File name: Image0037(Partial Open).bmp

File type: Bitmap (*.bmp)

Bit Depth 8 [Bits/Pixel]

Save the image files of each frame in the following new folder. "Sample_XYZT.jpg.frames¥"

● Selected ROI Shape (Select one or more in the following)

Output Format: Merge Channel (using assigned LUT)

Merge Method: Amount

☑ Gray Scale (after merging)

L Start 1 End 1 Step 1 1 / 2

Z Start 1 End 3 Step 1 3 / 3

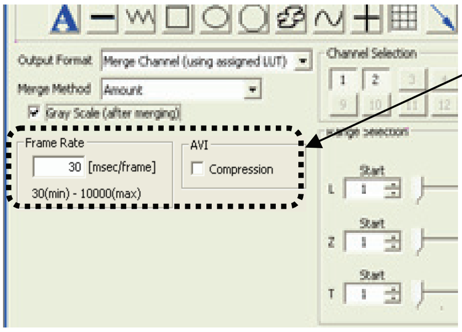
T Start 1 End 5 Step 1 5 / 5

Range selection
Specify the Start and End values of axes to be read (L/Z/T).

Saving images in movie format (AVI or MOV)

(The AVI or MOV format is available for XYZ, XYT, XYλ, XZT, XλT, and XλZ images.)

1. Open the Live View window or 2D View window of the image to be saved. Right-click on the image and then select Export.
2. Select a movie format and enter the frame rate in the Frame Rate field.
3. To save the file, click Save.


Channel Selection
1 2 3
9 10 11 12
Range selection
AVI

Output Format: Merge Channel (using assigned LUT)

Merge Method: Amount

☑ Gray Scale (after merging)

Frame Rate: 30 [msec/frame] 30(min) - 10000(max)

☐ Compression

L Start 1

Z Start 1

T Start 1

Frame Rate
The frame rate of the movie and whether to set compression or not can be set.
(AVI: Sets whether to compress the AVI file.)

Only for SPD

Adjustment of Acquisition Wavelength Range (VBF)

The wavelength range of waves acquired in Ch1 and Ch2 can be adjusted by using the **Variable Barrier Filter**.

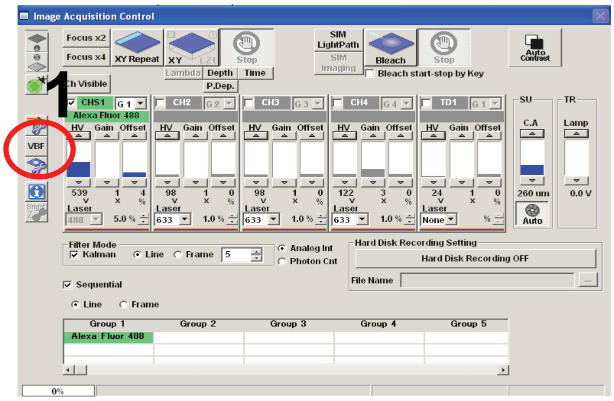
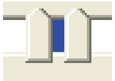
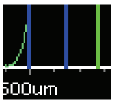
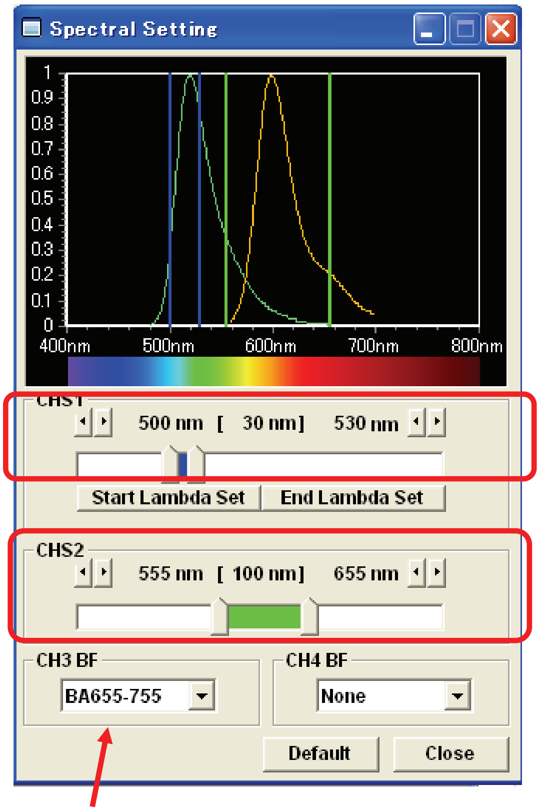


Image Acquisition Control
1
VBF

1. Click the VBF button to open the Spectral Setting window.

2. Using  or , move the position to adjust the wavelength of the wave to be acquired.

Spectral Setting window


Spectral Setting
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400nm
500nm
600nm
700nm
800nm
Start Lambda Set
End Lambda Set
Default
Close

CHS1: 500 nm [30 nm] 530 nm

CHS2: 555 nm [100 nm] 655 nm

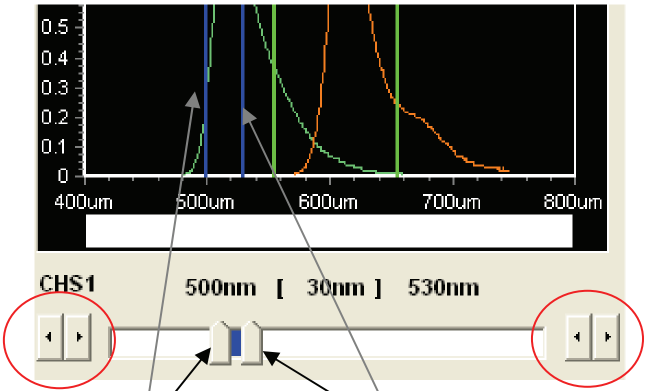
CH3 BF: BA655-755

CH4 BF: None

Slit adjustment for Ch1

Slit adjustment for Ch2

Fluorescent filter selection for Ch3


0.5
0.4
0.3
0.2
0.1
0
400um
500um
600um
700um
800um

CHS1: 500nm [30nm] 530nm

Changes the **slit position**.

Changes the **slit width**.

Changes the **slit position** in 1 nm increments.

Changes the **slit width** in 1 nm increments.

Hard Disk Recording

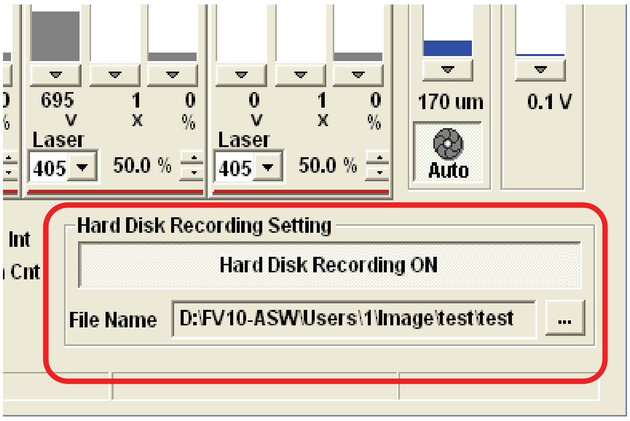
- This function automatically saves acquired images in a preset save destination.
- When series imaging is executed, you can check images up to the immediately preceding one through the Viewer window in the middle of the image acquisition process.

[Hard disk recording conditions]

Maximum number of image acquisition	32,766 times (number of images in series)
Save destination	Only local drives are guaranteed (D drive)
Other conditions	XYT images below 128×128 in size. Intervals of 1 second or more are required. Disabled for the reciprocating scan mode. **The review function is only available for interval operation.**

How to acquire images

1. Set the image adjustment and condition settings.
2. Click the Hard Disk Recording OFF button. The button appears as Hard Disk Recording On and the Viewer window opens.
3. Click the ... button next to the File Name textbox. Set the save destination and file name and then click Open.
4. To acquire images, click the XY LZt button.



Hard Disk Recording Setting

Hard Disk Recording ON

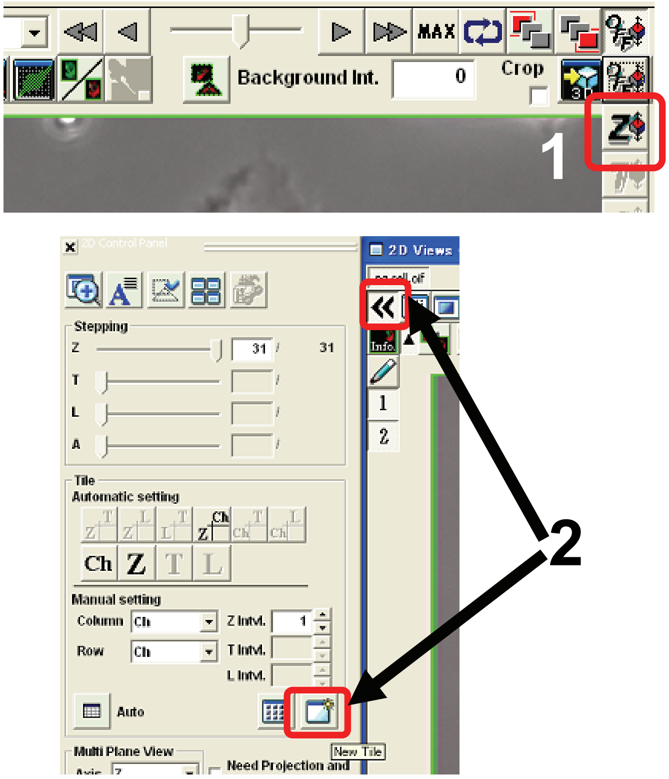
File Name D:\FV10-ASW\Users\1\Image\test\test ...

Viewing images in the middle of the image acquisition process

1. After image acquisition starts, bring the Viewer window to the front.
2. Double-click an image file during the acquisition process with the view set to Thumbnail in the Explorer window.

(When image files are not displayed in the Thumbnail view during the acquisition process, right-click and select Refresh.)

A. Creation of 2D Z Projection Images
B. Reloading Set Conditions

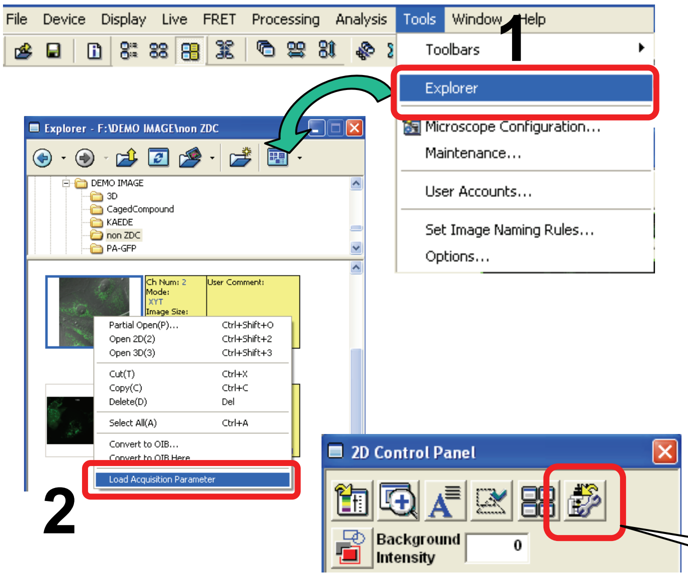

MAX
Background Int.
0
Crop
1
2D Control Panel
2D Views
Info.
Stepping
Z
31
31
T
L
A
Tile
Automatic setting
Ch
Z
T
L
Manual setting
Column
Ch
Z Intvl.
1
Row
Ch
T Intvl.
L Intvl.
Auto
New Tile
Multi Plane View
Need Projection and
2

A. Creation of 2D Z projection images

1. Open an original image and then select Z from the Projection button in the 2D View to open the Z projection image.

2. Click the New Tile button in the 2D Control Panel (opened by clicking «) to create a 2D image of the Z projection image.

3. Save the image.


File
Device
Display
Live
FRET
Processing
Analysis
Tools
Window
Help
Toolbars
Explorer
Microscope Configuration...
Maintenance...
User Accounts...
Set Image Naming Rules...
Options...
1
Explorer - F:\DEMO IMAGE\non ZDC
DEMO IMAGE
3D
CagedCompound
KAEDE
non ZDC
PA-GFP
Ch Num: 2
Mode: XYT
Image Size:
User Comment:
Partial Open(P)... Ctrl+Shift+O
Open 2D(2) Ctrl+Shift+2
Open 3D(3) Ctrl+Shift+3
Cut(T) Ctrl+X
Copy(C) Ctrl+C
Delete(D) Del
Select All(A) Ctrl+A
Convert to OIB...
Convert to OIB Here
Load Acquisition Parameter
2
2D Control Panel
Background Intensity
0

B. Reloading set conditions

Loads image acquisition conditions from past image data in order to duplicate them.

1. Select Explorer from Tools.

2. Right-click on a thumbnail image from which to reload set conditions, and then select Load Acquisition Parameter.

3. The conditions are reloaded.

Conditions can be reloaded by clicking the Load Scan Data button while the image from which to reload conditions is open.

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